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Comité Scientifique International de Recherches sur les Trypanosomiases

TENTH MEETING
DIXIEME REUNION

KAMPALA

1964

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ADDRESS GIVEN BY MR. A. O. ODELOLA, ACTING SECRETARY-GENERAL OF CCTA, TO THE ISCTR MEETING

Mr. Chairman, distinguished Delegates,

I regret that, owing to unavoidable circumstances, I was unable to be here for the opening ceremony last Tuesday.

I am grateful to the Government of Uganda for their kind hospitality and the wonderful facilities placed at the disposal of this meeting. I hope that, in the usual way, this meeting will, by the necessary resolution, express the sincere thanks of all the delegates for the helpful co-operation received from the Government of Uganda for this meeting.

As distinguished delegates are aware, the CCTA, with which your Council is in active co-operation, will become an organ of the OAU with effect from 1 January 1965, following the adoption by the Heads of State of African countries of a proposal drawn up by the Acting Secretary-General of the CCTA and the provisional Secretary-General of the OAU, as follows:

(*a*) 31 December 1964 should be the deadline for bringing about complete integration.

(*b*) To this end, a firm resolution either by the Council or the Assembly of Heads of State and Government be passed declaring that the functions so far exercised by the CCTA should now be assumed by the Scientific, Technical and Research Commission of the OAU. Such other functions exercised by the CCTA and not falling directly within the terms of reference of the Scientific, Technical and Research Commission as recommended by the latter and as approved by the Council, should be transferred to the competent and appropriate Specialised Commissions of the OAU.

(*c*) In the face of the withdrawal, as from December 1963, of financial support by the European members of the CCTA for the administrative budget of that organisation, a special call should be made on all African members of the CCTA to pay in their contributions to the budget in order to ensure continuity of the activities of the CCTA up to 31 December 1964, the date set for integration.

(*d*) As from 1 January 1965, the budget of the CCTA become part of the consolidated budget of the OAU and apportioned among all thirty-four members of the OAU in accordance with the scale of assessment of the OAU. As the Council of Ministers' budget session is to be held in February of next year, the incoming Secretary-General be authorised to advance money for the financing of the continuation of the activities of the CCTA between 1 January 1965 and the approval of the OAU budget.

(*e*) Planning should immediately be started by the OAU and the CCTA secretariats with a view to extending as from 1 January 1965 the activities of the CCTA to all OAU countries. On the CCTA side much of this planning could be accomplished by the Extraordinary Session of the Administrative Committee, due to convene some time in October 1964. On the OAU side, the visit of African scientists to the various bureaux of the CCTA envisaged by the recommendations of the Scientific, Technical and Research Commission, also adopted by the Council, could assist the

Secretary-General in evaluating the programmes of the CCTA and in planning their extension to all members of the OAU. Furthermore, the programmes and the activities of the CCTA for 1965 be evaluated by the forthcoming session of the Scientific, Technical and Research Commission of the OAU whose meeting could be timely if it were to be held some time in December 1964—in the context of the terms of reference of the Commission as well as its overall programme of work.

(*f*) The question of relocating the institutions of the CCTA to the headquarters of the OAU be left to the consideration of the forthcoming session of the Scientific, Technical and Research Commission.

(*g*) The existing staff of the CCTA constitute the nucleus of the Secretariat of the Scientific, Technical and Research Commission, it being understood that such officers as are presently in the service of the CCTA convert individually to the terms of employment of OAU or complete their present contract on existing terms.

(*h*) Integration implies that the OAU will take over the assets and the liabilities of the CCTA by 1 January 1965. In this respect the Secretary-General of the OAU with the Secretariat of the CCTA recommend to the forthcoming session of the Scientific, Technical and Research Commission a formula by which existing members of the CCTA will be compensated by the other members of the OAU.

In keeping with this declaration I summoned an Extraordinary Meeting of the Administrative Committee of the CCTA and obtained from the Committee suggestions and ideas for the next stage of the exercise, namely the actual practical ways and means of ensuring a smooth transition from the old CCTA to the new Scientific Commission of the OAU.

The meeting was attended by Mr. Diallo Telli, the Secretary-General of the OAU, who also impressed on the Committee the need to make a critical review of the activities of the Organisation as at present constituted and also to make suggestions for the future expansion of its activities within the family of the OAU.

Where then does the ISCTR come in? Mr. Chairman, your Council is an important one, judging from its previous activities in stimulating and encouraging discussions and research on both human and animal trypanosomiasis. The Specialist Meeting in Lagos recommended among other things that your Council should review its role in the context of present-day development in Africa so as to be better able to render valuable assistance to national organisations concerned with trypanosomiasis. To this end, you were to review your present constitution, change the name of your organisation from "Committee" to "Council", set up an Executive Committee to supervise the activities of your permanent office, and set up a Permanent Bureau for trypanosomiasis research with a Director for the Bureau. He will be in active contact with national and international organisations and he will ensure the proper dissemination of information in this specialised field.

As you are aware, Mr. Chairman, circumstances beyond our control have delayed the execution of most of the urgent suggestions contained in the recommendations of the Expert Meeting which met in Lagos. At present, we are in a position to discuss these issues and make suggestions as to what should be done when action is finally taken.

I regret, Mr. Chairman, that the time is not opportune for a final decision on

the establishment of permanent bodies, because the terms of reference of your Council embrace both human and animal trypanosomiasis.

As I have outlined above, the CCTA will become the Scientific, Technical and Research Commission of the OAU. At the same time, the OAU has another Commission dealing with nutrition, health and sanitation. In view of this, it would appear that the terms of reference of your Council impinge on the scope of activities of these two Commissions. As for the permanent organs of the CCTA which are already established and whose functions do not fall within the scope of the STRC, it is intended that these would be transferred eventually to appropriate Commissions of the OAU. In view of this, establishment of new organs whose functions are likely to cut across the terms of reference of more than one Commission of the OAU, should wisely be postponed till a future date so as not to prejudice future decisions. However, it is my intention that your Council will continue to be associated with the Scientific, Technical and Research Commission of the OAU until definite decisions are taken as to which Commission should absorb its activities. Because of this, it will be helpful to our thinking at the Secretariat if concrete suggestions could be given by this Council on the need to set up the Trypanosomiasis Bureau, possible location of the Bureau, and an idea as to the type of machinery required to bring it into operation.

Some time in May this year, the Secretariat received a suggestion from the ECA on the possibility of launching a trypanosomiasis eradication campaign on a regional basis almost on similar lines to the joint campaign for the eradication of rinderpest or the locust eradication campaign.

As distinguished delegates are aware, your Council, at its Ninth Meeting, suggested the setting up by the CCTA Secretariat of the Kissi survey team, which has now completed its work and submitted its report.

I hope that, as a result of your deliberations, it may be possible, using the recommendations of the Kissi mission as a springboard, to direct thoughts to the possible eradication campaign on a regional basis in Africa.

Finally, I thank you very much, Mr. Chairman, for giving me this opportunity of addressing distinguished scientists here present.

I wish you success in your deliberations.

DISCOURS PRONONCE PAR M. A. O. ODELOLA, SECRETAIRE GENERAL p.i. DE LA CCTA A LA REUNION DU CSIRT

Monsieur le Président,
Honorables délégués,

Je regrette que les circonstances ne m'aient pas permis de me trouver présent à la cérémonie d'ouverture, mardi dernier.

Je suis reconnaissant au Gouvernement de l'Ouganda de son aimable hospitalité et des excellentes facilités qui ont été mises à la disposition de la réunion. J'espère qu'une résolution exprimera les remerciements de tous les délégués pour la coopération efficace reçue du Gouvernement de l'Ouganda à l'occasion de cette réunion.

Comme le savent les honorables délégués, la CCTA, avec laquelle votre Conseil coopère activement, deviendra une institution de l'OUA à partir du 1er janvier 1965, à la suite de l'adoption par les Chefs d'Etat des pays africains d'une proposition présentée par le Secrétaire Général p.i. de la CCTA et le Secrétaire Général provisoire de l'OUA ainsi qu'il suit:

a) On pourrait arrêter au 31 décembre 1964 le délai à l'issue duquel l'intégration devra être complètement réalisée.

b) A cet effet, le Conseil ou la Conférence des Chefs d'Etat et de Gouvernement devront formuler une résolution décisive déclarant que les fonctions exercées jusqu'à présent par la CCTA seront transférées à la Commission Scientifique, Technique et de la Recherche de l'OUA. Toutes fonctions exercées éventuellement par la CCTA qui seraient étrangères au mandat de la Commission Scientifique, Technique et de la Recherche, tel que recommandé par ladite Commission et approuvé par le Conseil, seront transférées aux Commissions spécialisées compétentes de l'OUA.

c) En présence de la suppression depuis décembre 1963 du soutien financier accordé par les membres européens de la CCTA au budget administratif de l'organisation, il y aura lieu de lancer un appel particulier à tous les membres africains pour qu'ils versent leur contribution au budget afin que les activités de la CCTA puissent se poursuivre jusqu'au 31 décembre 1964, date fixée pour l'intégration.

d) A partir du 1er janvier 1965, le budget de la CCTA sera intégré au budget global de l'OUA; les trente-deux membres de l'OUA y contribueront conformément au barême des cotisations de l'OUA. Comme la session du Conseil des Ministres qui aura à approuver le budget doit avoir lieu en février 1965, il conviendra que le Secrétaire Général titulaire soit autorisé à avancer les crédits qui permettront à la CCTA de poursuivre ses activités entre le 1er janvier 1965 et le moment où le budget de l'OUA sera approuvé.

e) Le Secrétariat de l'OUA et celui de la CCTA devront entreprendre immédiatement d'établir les plans qui permettront, à partir du 1er janvier 1965, d'étendre les activités de la CCTA à tous les pays membres de l'OUA. En ce qui concerne la CCTA, une grande partie de cette planification pourra être entreprise à l'occasion de la session extraordinaire du Comité administratif qui doit se réunir en octobre 1964. Pour l'OUA, la visite de

scientifiques africains aux divers bureaux de la CCTA proposée dans les recommandations de la Commission Scientifique, Technique et de la Recherche et adoptée ensuite par le Conseil, pourra être utile au Secrétaire Général pour l'examen critique des programmes de la CCTA et la planification de leur extension à tous les membres de l'OUA. D'autre part, les programmes et les travaux de la CCTA pour 1965 devront être examinés à l'occasion de la prochaine session de la Commission Scientifique, Technique et de la Recherche; il serait opportun que cette réunion ait lieu en décembre 1964, conformément aux termes du mandat de la Commission et de son programme général de travail.

f) Il y aura lieu de laisser à la Commission Scientifique, Technique et de la Recherche, lors de sa prochaine session, le soin d'examiner la question du transfert des institutions de la CCTA au siège de l'OUA.

g) Le personnel actuel de la CCTA devra constituer le noyau de la Commission Scientifique, Technique et de la Recherche, étant entendu que les fonctionnaires actuellement au service de la CCTA pourront accepter individuellement les conditions d'emploi de l'OUA ou terminer leur contrat conformément aux conditions qu'il stipule.

h) L'intégration implique la reprise par l'OUA, au 1er janvier 1965, de l'actif et du passif de la CCTA. A cet égard, le Secrétaire Général de l'OUA et le Secrétariat de la CCTA recommandent à la Commission Scientifique, Technique et de la Recherche d'arrêter lors de sa prochaine session une formule permettant aux membres de l'OUA d'indemniser les membres actuels de la CCTA.

Conformément à cette déclaration, j'ai convoqué une réunion extraordinaire du Comité Administratif de la CCTA et obtenu de ce Comité des suggestions et des idées pour l'accomplissement de la phase suivante, à savoir les voies et moyens pratiques permettant d'assurer une transition sans heurts de l'ancienne CCTA à la nouvelle Commission Scientifique de l'OUA.

M. Diallo Telli, Secrétaire Général de l'OUA, assistait à la réunion; il a bien fait comprendre au Comité la nécessité d'effectuer un examen critique des activités de l'Organisation telle qu'elle est à présent constituée et en même temps de faire des suggestions pour l'expansion future de ses activités au sein de la famille de l'OUA.

Et l'ISCTR? Monsieur le Président, votre Conseil est très important, à en juger par ses activités passées pour stimuler et encourager les discussions et la recherche dans le domaine de la trypanosomiase tant humaine qu'animale. La réunion de spécialistes de Lagos a recommandé, entre autres choses, que votre Conseil revoie son rôle dans le contexte des développements qui prennent actuellement place en Afrique, afin d'être mieux en mesure de fournir une aide précieuse aux organisations nationales s'intéressant aux trypanosomiases. Dans ce but, vous deviez revoir votre constitution actuelle, changer le nom de votre Organisation de " Comité " en " Conseil ", nommer un Comité Exécutif pour superviser les activités de votre siège, établir un bureau permanent pour la recherche sur les trypanosomiases à la tête duquel se trouverait un Directeur. Il serait en contact avec les organisations nationales et internationales et assurerait la diffusion correcte des renseignements dans ce domaine spécialisé.

Comme vous le savez, Monsieur le Président, des circonstances indépendantes

de notre volonté ont retardé la mise en œuvre de la plupart des suggestions urgentes contenues dans les recommandations de la réunion d'experts qui s'est réunie à Lagos. A l'heure actuelle, nous sommes à même de discuter de ces questions et de faire des suggestions quant à ce qui devrait être fait lorsque des mesures seront enfin prises.

Je regrette, Monsieur le Président, que le moment ne soit pas opportun pour décider de l'établissement d'organismes permanents, le mandat de votre Conseil embrassant tant la trypanosomiase humaine qu'animale.

Comme je viens de le signaler, la CCTA deviendra la Commission Scientifique, Technique et de la Recherche de l'OUA. En même temps, l'OUA a une autre Commission traitant de la nutrition, de la santé et de l'hygiène. Il semblerait donc que le mandat de votre Conseil empiète sur le champ d'activités de ces deux Commissions. En ce qui concerne les organismes permanents de la CCTA, qui sont déjà établis et dont les fonctions ne sont pas de la compétence de la CSTR, on a l'intention de les transférer par la suite aux Commissions appropriées de l'OUA. Etant donné ceci, il semble donc sage de reporter à une date ultérieure l'établissement de nouveaux organismes dont les fonctions empièteront vraisemblablement sur le mandat de diverses Commissions de l'OUA, afin de ne pas nuire aux décisions futures. Toutefois, mon intention est que votre Conseil continue à être associé à la Commission Scientifique, Technique et de la Recherche de l'OUA jusqu'à ce que des décisions définitives soient prises quant à la Commission qui devrait absorber ses activités. Il serait utile que le Secrétariat reçoive du Conseil des suggestions concrètes quant à la nécessité d'établir le Bureau des Trypanosomiases, l'emplacement éventuel de ce Bureau, et une idée quant au type d'organisation nécessaire à son fonctionnement.

En mai cette année, la CEA a suggéré la possibilité de lancer une campagne d'éradication contre la trypanosomiase sur une base régionale, presque sur les mêmes lignes que la campagne conjointe pour l'éradication de la peste bovine ou la campagne pour l'éradication du criquet.

Comme le savent les honorables délégués, votre Conseil, lors de sa 9e réunion, a suggéré l'établissement par le Secrétariat de la CCTA d'une équipe d'enquête dans la région Kissi; celle-ci a maintenant terminé ses travaux et soumis son rapport.

J'espère qu'à l'issue de vos délibérations il pourra être possible, en utilisant les recommandations de la mission Kissi comme tremplin, de diriger les pensées vers une éventuelle campagne d'éradication sur une base régionale en Afrique.

Je vous remercie enfin, Monsieur le président, de m'avoir permis de m'adresser aux honorables hommes de science ici présents.

Je vous souhaite le succès dans vos travaux.

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- 1.2. Diagnosis, Immunology and Biochemistry.
- 1.3. Protozoology.

2. Entomology

- 2.1. Biology of *Glossina*.
- 2.2. Control of *Glossina*.
  - 2.2.1. By methods of indirect attack: clearing, game elimination, chemosterilisation.
  - 2.2.2. By direct attack: insecticidal methods.

3. Human trypanosomiasis

- 3.1. Epidemiology.
- 3.2. Chemotherapy.
- 3.3. Diagnosis.
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4. Consideration of the recommendations of the Meeting of Experts held in Lagos 25-28 September 1963

- 4.1. Recommendation V: Redesignation of ISCTR as the International Scientific **Council** for Trypanosomiasis Research (**Conseil** Scientifique International de Recherches sur les Trypanosomiases).
- 4.2. Recommendation VI: Extension of the scope of ISCTR.
- 4.3. Recommendation VII: Executive Committee of ISCTR.
- 4.4. Recommendation VIII: African Trypanosomiasis Bureau.
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5. Election of Chairman and Executive Committee

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1.2. Diagnostic, immunologie et biochimie.

1.3. Protozoologie.

2. Entomologie

2.1. Biologie des glossines.

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2.2.1. Par voie d'attaque indirecte: débroussaillement, destruction du gibier, chimiostérilisation.

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3. Trypanosomiase humaine

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3.2. Chimiothérapie.

3.3. Diagnostic.

3.4. Protozoologie.

4. Examen des recommandations de la Réunion d'Experts tenue à Lagos du 25 au 28 septembre 1963

4.1. Recommandation V: Modification de l'appellation du CSIRT, qui sera désormais le " **Conseil** Scientifique International de Recherches sur les Trypanosomiases " (International Scientific **Council** for Trypanosomiasis Research).

4.2. Recommandation VI: Elargissement du champ d'activité du CSIRT.

4.3. Recommandation VII: Comité Exécutif du CSIRT.

4.4. Recommandation VIII: Bureau Africain des Trypanosomiases.

4.5. Autres questions découlant des recommandations de la Réunion d'Experts.

5. Election du Président et du Comité Exécutif

REPORTS

ITEM 1.—ANIMAL TRYPANOSOMIASIS

Rapporteurs: Dr. P. Finelle
Mr. M. P. Cunningham
Mr. M. J. Hope Cawdery

1.1. Epizootiology, chemotherapy and drug resistance

(*a*) The Council noted that there has been little progress in new approaches to the chemotherapy of animal trypanosomiasis. It is hoped that research on new prophylactic drugs will be started again with particular reference to new chemical groups.

(*b*) It would appear that the prophylactic drugs can be used to protect donkeys against *T. congolense* group and *T. vivax* group infection. In areas where *T. brucei* group organisms are present protection may be reduced.

(*c*) It was noted that Berenil is not excreted from treated animals as quickly as was previously thought. In discussion it was considered that this was to be expected from its relationship to established chemoprophylactic drugs. The use of Berenil for the treatment of human trypanosomiasis should be investigated further. It was considered that the technique used for estimating low levels of Berenil in the serum of treated cattle may have wide application. It is recommended that this technique be applied to other drugs.

(*d*) It was noted that in the use of prophylactic drugs there was little relationship between dosage rates and injection routes and the protective period.

(*e*) Possible reasons for the development of drug resistance in trypanosomes during chemoprophylaxis were noted and some possible solutions to prevent this were discussed.

1.2. Diagnosis, immunology and biochemistry

(*a*) Present techniques are sufficiently accurate for the diagnosis of cattle trypanosomiasis on a herd basis, but it was considered that more accurate techniques are required for research purposes.

(*b*) It was noted that basic antigenic types are associated with strains of *T. brucei* group organisms on cyclical or mechanical transmission.

It was considered that other serological techniques, including a neutralisation test, would be valuable in further investigations on this subject.

(*c*) Cattle infected with antigenic variants of the *T. brucei* subgroup and treated with Berenil three days after infection, produce high titre homologous agglutinins. It would appear that on inoculation of several antigenic variants simultaneously into an ox, agglutinins against all inoculated variants are subsequently produced.

(*d*) It is recommended that serological investigations using *T. congolense* and *T. vivax* groups be carried out.

1.3. Protozoology

(*a*) It was noted that while organisms of the *T. brucei* group may be separated from blood using differential centrifugation, further investigations are required for *T. congolense* and *T. vivax* group organisms.

(*b*) It was considered that inoculation of triturated *Glossina* into mice was a valuable method for isolation of trypanosomes from wild caught *Glossina*.

(*c*) Preliminary investigations on the metabolism of *T. evansi* in culture were noted with interest.

ITEM 2.—ENTOMOLOGY

Rapporteurs: Mr. A. Challier
Dr. G. F. Burnett
Mr. J. M. B. Harley
Dr. E. J. Buyckx
Dr. P. E. Glover

Reports of recent work on *Glossina* were considered by the Council under two headings: the biology of the vector; and recent attempts at control, the majority by means of insecticides.

2.1. Biology of *Glossina*

Seasonal and annual variations in the size of *Glossina* populations are important to the timing and to the evaluation of control methods. The first sixteen years' data from a long-term study in Northern Nigeria of *G. palpalis* show that fluctuations from one year to another are small compared with those of other animal populations.

Seasonal changes in the physiological age of female *G. fuscipes* in Uganda were closely correlated with changes in infection rate. Mean age and infection rate both increased during the wet seasons and decreased in the dry weather. It was demonstrated that the method for determining the physiological age of female *Glossina* could be extended so that older flies are more accurately aged, thus increasing considerably the value of the method.

2.2. Control of *Glossina*

Results of preliminary experiments on the sterilisation of *Glossina* by means of both chemicals and gamma irradiation are promising and it seems that it will soon be possible to devise a practical method for sterilising males. Techniques for the mass rearing of *G. morsitans* in large cages in the field are being investigated with a view to providing the large numbers required if sterilisation is to be used as a method of control.

Papers presented reported the successful attack on *Glossina* spp. using insecticides by two different methods, the single or once repeated application of residual insecticides to the resting places of the fly and the repeated dissemination of aerosols from ground or air to kill by direct contact without residual effect. Both methods are becoming increasingly selective.

Treatment of resting places has been successful against *Glossina* of both the *morsitans* and *palpalis* groups and can be considered a proven reliable method, provided there is adequate information on the biology of the species under attack and the qualities of the insecticides used, in the locality concerned. Aerial spraying recently has been confined to attack on savannah *Glossina* in East Africa and has been used successfully and economically on a large scale in Rwanda. Since the techniques used there were elaborated, research has reduced costs by some 50%. Increased efficiency is forecast which will reduce environmental contamination with insecticide applied from the air.

Various methods of control have their place according to circumstances and they should be used in combination. There is no universal " best method ".

Such observations as have been made so far show little confirmed effect of chemical control on wild life, and any such effect is likely to be transient and less marked than that caused by alternative methods involving modification of the environment.

The Council strongly emphasises that reclamation, especially by chemical methods, should only be undertaken as part of a complete development scheme for the land affected and that the timetable for the whole scheme must be followed if the benefit of controlling *Glossina* is not to be lost. The cost of anti-tsetse measures should be considered in relation to the cost of the whole development scheme and not in isolation. In order to halt epidemics of trypanosomiasis or tsetse advances special measures may be necessary.

ITEM 3.—HUMAN TRYPANOSOMIASIS

Rapporteurs: Médecin-Général-Inspecteur P. Richet
Dr. R. J. Onyango
Dr. H. J. C. Watson
Dr. P. De Raadt

3.1. Epidemiology

3.1.1. Information on endemicity, recrudescence or new outbreaks

Professor van den Berghe presented a paper on a focus of *T. rhodesiense* infection in Burundi. A flare-up appears to be developing north of Muhinga, which is comparable to the present epidemics occurring round the shores of Lake Victoria.

Dr. Burke discussed the human trypanosomiasis position in certain regions of the Congo Leopoldville. He pointed out that early cases had become rare and that most cases now presented mental symptoms. The great danger of the situation in this country was shown by the figures he gave; in some places along the Angola border the infection rate had reached 3 to 6%.

Médecin-Général-Inspecteur Richet gave a paper which set out clearly the satisfactory situation in French-speaking West Africa. This paper also contained valuable information on HB_2M (elevated levels of the I_gM class of immunoglobulins in the serum) used for diagnosis, and a report on treatments used in the various member countries of OCCGE.

3.1.2. Mechanisms of maintenance and spread of trypanosomiasis: reservoirs of infection, conditions of man-fly contact, movements of infected persons, etc.

It has been shown that when a residual focus of trypanosomiasis is badly or irregularly controlled there can remain in the area a source of infection which can develop into a local epidemic which may then spread throughout the area.

It has also been shown that when the infection rate in an area rises to a high level, the danger of trypanosomiasis can be eliminated only by the introduction of specialised teams to control the outbreak.

When human sleeping sickness due to either *T. gambiense* or *T. rhodesiense* is found in an area, every effort must be made to control it, using all methods available:

—Complete surveys should be carried out at least once, and preferably twice, yearly.
—Whenever possible serum should be examined for HB_2M; this should also be looked for in CSF. In patients with HB_2M every effort must be made to find trypanosomes, by conventional methods repeated as often as necessary.
—According to circumstances selective or mass chemoprophylaxis should be carried out.
—Vector control should be carried out, using insecticides, by modification of the vegetation, or by land development.
—An adequate check should be kept on the itinerant populations.

Particular importance is attached to interterritorial surveys such as that just carried out by CCTA and OCCGE with financial assistance from U.S. AID in the Kissi area which extends into Guinea, Sierra Leone and Liberia.

3.2. Chemotherapy

Dr. Hawking produced a paper which set out a hypothesis for the mechanism of the acquisition of arsenical resistance in trypanosomes.

Dr. Watson presented a final follow-up report on the use of Mel.W. in Northern Nigeria, suggesting that it had a place in the chemotherapy of *T. gambiense* sleeping sickness.

Médecin-Général-Inspecteur Richet produced figures from OCCGE which showed the great value of Mel.B. when used in the regime described by Neujean.

3.3. Diagnosis

Work done by Dr. Onyango and Dr. De Raadt has confirmed the difficulty of diagnosis of trypanosomiasis in periods of low parasitaemia, the reappearance of trypanosomes in phases of high parasitaemia and, because of these, the need for long-term observations of patients to confirm the presence of trypanosomes.

Dr. Lumsden gave an excellent dissertation on the use of tests for HB_2M and pointed out some instances where it could give a misleading result. He pointed out that HB_2M should be taken as an indication that trypanosomiasis is a probable diagnosis but not a firm one.

3.4. Protozoology

Dr. Willett described some features of the present outbreak of *T. rhodesiense* transmitted by *G. fuscipes* in Alego location, Central Nyanza, Kenya, and put forward a hypothesis which, while explaining the general association of *T. gambiense* with *G. palpalis* group flies and *T. rhodesiense* with *G. morsitans* group flies, made it clear that there was no reason why either trypanosome species should not be transmitted, under suitable circumstances, by either group of flies.

Professor van den Berghe also expressed some views about the relations between *T. gambiense* and *T. rhodesiense* and the present and future passage of *T. rhodesiense* through the ecological barrier between the savannah and the forest.

RECOMMENDATIONS

I. MESSAGE OF THANKS

The Council (see Recommendation II) THANKS the Government of Uganda for their generous hospitality. It also EXPRESSES its gratitude to the Dean and the Faculty of Medicine of Makerere University for providing accommodation and facilities for the meeting and to the Commissioner of Veterinary Services, Uganda, and his assistants for all their help in the organisation of the meeting.

II. REDESIGNATION OF ISCTR

The Committee RECOMMENDS that it shall, in future, be designated the International Scientific Council for Trypanosomiasis Research.

III. RELATIONSHIP OF ISCTR TO OTHER ORGANISATIONS

The Council CONSIDERS that its activities would be best promoted by its continued independent existence. It should continue its association with CCTA, and later with the appropriate Commissions of OAU; it should also collaborate closely with WHO and FAO.

IV. EXTENSION OF SCOPE OF ISCTR

The Council ENDORSES Recommendation VI of the Meeting of Experts in Lagos, September 1963, and considers that everything possible should be done to ensure that research workers in Africa should be enabled to keep in touch with research being conducted elsewhere in the field of trypanosomiasis. Moreover, the gap which has tended to exist between workers on this disease and on the tsetse fly and research workers in other fields whose results may be of importance to research on trypanosomiasis should be narrowed.

Furthermore, it is clearly desirable that all international organisations concerned with trypanosomiasis should be fully associated with ISCTR.

The Council therefore RECOMMENDS that its constitution should be that of the previous Committee enlarged on the following lines:

(1) Membership of the Council should be offered as of right to a representative of any international organisation working in the field of trypanosomiasis.

(2) Directors of the institutes in Africa having a programme on trypanosomiasis research, or one representative of each, should be entitled to membership on the invitation of the Executive Committee of the Council.

(3) The Council should have power to co-opt for all scientific discussions members from other countries where research on trypanosomiasis or related subjects is being carried out.

V. THE EXECUTIVE COMMITTEE

The Council RECOMMENDS that it should have an Executive Committee consisting of:

(1) The Chairman of ISCTR.

(2) Four other acknowledged experts, whose experience should cover as many as possible of the different disciplines concerned, selected by ISCTR.

(3) One representative each from WHO, FAO and any other organisation making substantial financial contribution, if invited by ISCTR.

The following at least should be included in the functions of the Committee:

(1) To act as a Steering Committee for ISCTR.

(2) To convene *ad hoc* meetings of specialists when required.

(3) To act as an advisory body on:

(*a*) priorities in research and control programmes;

(*b*) support for research on trypanosomiasis;

(*c*) the allocation of funds;

(*d*) the initiation of new lines of research.

(4) To be responsible to the Council and to report to it at regular intervals.

The Committee should have an annual meeting and should meet more often as required. Financial provision in the first instance should be made for two meetings a year.

The Council CONSIDERS it desirable that meetings of the Committee should be held in turn in the several regions of Africa where trypanosomiasis exists.

VI. THE PROPOSED AFRICAN TRYPANOSOMIASIS BUREAU

The Council CONSIDERS that in view of the establishment of an Executive Committee of the Council and of the establishment of an information service on trypanosomiasis by WHO, the decision on the setting up of an African Trypanosomiasis Bureau should be postponed until it becomes clear whether such a Bureau is needed.

VII. THE COUNCIL'S REPORT

The Council CONSIDERS that the full reports of its meetings, embodying the scientific papers and the summaries of discussions on them, are of great value to research workers in the field of trypanosomiasis and RECOMMENDS that the report of the present meeting be published at the earliest possible date.

It REQUESTS that reprints should be made available to the authors of the scientific papers.

VIII. STANDARDISATION OF COSTINGS OF CONTROL OPERATIONS

The Council RECOMMENDS that reports on the cost of tsetse control operations with insecticides should be standardised in the following form:

A. Capital expenditure

—Vehicles and accessories (e.g. trailers)—type and price.

—Spraying equipment—type and price.

—Facilities (buildings, camp equipment, depots, landing strips, mobile workshops, etc.).

—Any other equipment.

B. Operating costs

	Preliminaries		*Campaign*		*Maintenance*	
	Quantity	*Cost*	*Quantity*	*Cost*	*Quantity*	*Cost*
I. Staff						
(*a*) Scientific						
(*b*) Technical and Field						
(*c*) Transport of Staff						
(*d*) Central Administration						
II. (i) Spraying						
(*a*) Equipment						
(*b*) Insecticides						
(*c*) Diluents						
(*d*) Miscellaneous						
(ii) Transport of above						
(*a*) Equipment						
(*b*) Insecticides						
(*c*) Diluents						
(*d*) Miscellaneous						
III. Supplementary operations (Isolation, barriers, etc.)						
IV. Facilities (Buildings, camp equipment, depots, landing strips, workshops, access roads, etc.)						
V. Number of linear km. (miles) or sq. km. (square miles) treated. Cost per unit of area or linear unit treated.						

C. Estimate of recoverable costs of capital equipment

Definitions of terms employed

Preliminaries include:

—preliminary surveys,
—aerial photographs and mapping,
—reconnaissance of the site,
—biological assessment,
—administrative co-ordination,
—planning,
—ordering,
—transporting and supervision of equipment and supplies.

Maintenance includes cost of all measures necessary to maintain and consolidate the results of the operation.

Costs should show:

I. (*a*) Overall cost for the period.
(*b*) For contract staff, monthly rates; for labourers, man-days.
(*c*) Total cost.

II. Inclusive cost of equipment, accessories, spares, maintenance.

It should be clearly stated whether the cost per unit of area freed from infestation refers to permanent habitat or to the maximum dispersal area of the fly.

RAPPORTS

POINT 1. — TRYPANOSOMIASES ANIMALES

Rapporteurs: Dr P. Finelle
M. M. P. Cunningham
M. M. J. Hope Cawdery

1.1. Epizootiologie, chimiothérapie et résistance aux trypanocides

a) Le Conseil note que peu de progrès ont été réalisés dans le domaine de la chimiothérapie des trypanosomiases animales. Il souhaite que les recherches reprennent sur de nouveaux médicaments prophylactiques et, en particulier, sur ceux appartenant à de nouveaux groupes chimiques.

b) Les médicaments prophylactiques semblent pouvoir être utilisés pour protéger les ânes contre les infections dues aux sous-groupes *T. congolense* et *T. vivax*. Dans les régions où les organismes du groupe *T. brucei* sont présents, il se pourrait que la durée de protection soit réduite.

c) Le Bérénil n'est pas éliminé par les animaux traités aussi rapidement qu'on ne le pensait. Les discussions ont montré que ce fait était prévisible étant donné les relations avec d'autres médicaments prophylactiques usuels. L'emploi du Bérénil pour le traitement de la trypanosomiase humaine devra être étudié plus avant. La technique utilisée pour la mise en évidence de faibles concentrations de Bérénil dans le sérum des animaux traités pourrait recevoir une application plus étendue. Il est recommandé que cette technique soit appliquée aux autres médicaments.

d) Dans l'emploi des médicaments prophylactiques la dose utilisée et la voie d'injection n'ont que peu de rapports avec la durée de la protection.

e) La discussion porte sur l'apparition de la chimio-résistance chez les trypanosomes au cours des traitements chimio-préventifs, ses causes et les moyens éventuels de prévenir ce phénomène.

1.2. Diagnostic, immunologie et biochimie

a) Les techniques actuelles sont suffisamment précises pour le diagnostic de la trypanosomiase à l'échelle du troupeau, mais insuffisantes pour les besoins de la recherche.

b) Les types antigéniques de base sont associés à des souches du sous-groupe *T. brucei* à l'occasion de la transmission cyclique ou mécanique. D'autres techniques sérologiques, dont le test de neutralisation, seraient précieuses pour des recherches plus poussées.

c) Les bovins infectés avec des variants antigéniques appartenant au sous-groupe *T. brucei* et traités au Bérénil 3 jours après l'infection, produisent un titre élevé d'agglutinines homologues. Il semble que l'inoculation simultanée à un bovin de plusieurs variants antigéniques ait pour conséquence la production d'agglutinine contre l'ensemble des variants inoculés.

d) Il est recommandé que des recherches sérologiques avec *T. congolense* et *T. vivax* soient entreprises.

1.3. Protozoologie

a) Tandis que les trypanosomes du sous-groupe *T. brucei* peuvent être séparés du sang par centrifugation différentielle, d'autres recherches sont encore nécessaires pour *T. congolense* et *T. vivax*.

b) L'inoculation à la souris de glossines broyées constitue une bonne méthode pour l'isolement des trypanosomes à partir des glossines sauvages.

c) Le Conseil note avec intérêt les recherches préliminaires effectuées sur le métabolisme de *T. evansi* en culture.

POINT 2. — ENTOMOLOGIE

Rapporteurs: M. A. Challier
Dr G. F. Burnett
M. J. M. B. Harley
Dr E. J. Buyckx
Dr P. E. Glover

Le Conseil entend les rapports sur les travaux récents portant sur la biologie des glossines et sur les essais récents de lutte contre les vecteurs au moyen d'insecticides.

2.1. Biologie des glossines

Les fluctuations annuelles et saisonnières de populations de glossines constituent un facteur important dans le choix de l'époque du traitement et dans l'appréciation des méthodes de lutte. Selon les données recueillies au cours d'une période de 16 années en Nigéria du Nord sur *G. palpalis* les fluctuations annuelles sont faibles comparées à celles d'autres populations animales.

En Ouganda, les variations saisonnières de l'âge physiologique des femelles de *G. fuscipes* sont en étroite corrélation avec les variations du taux d'infection. L'âge moyen et le taux d'infection augmentent tous deux pendant la saison des pluies et diminuent à la saison sèche. Il a été démontré que la méthode de détermination de l'âge physiologique des femelles de glossines pouvait être étendue à l'estimation plus précise de l'âge des mouches âgées, ce qui peut augmenter considérablement l'intérêt de cette méthode.

2.2. Lutte contre les glossines

Les résultats des premières expériences de stérilisation des glossines, soit au moyen de produits chimiques, soit au moyen de radiations gamma sont prometteurs; il semble qu'une méthode pratique de stérilisation des mâles puisse bientôt être mise au point. Des techniques d'élevage intensif de *G. morsitans* dans de grandes cages, en brousse, sont à l'étude afin d'obtenir les quantités de mouches nécessaires à une généralisation de la lutte par stérilisation.

Les communications font état de campagnes concluantes contre les diverses espèces de glossines, en utilisant les insecticides selon deux méthodes. La première consiste en une ou deux applications d'insecticides rémanents aux endroits où se reposent les mouches. La seconde prévoit l'émission répétée d'aérosols à partir du sol ou par voie aérienne pour la destruction immédiate par contact direct, sans effet rémanent. Ces deux méthodes deviennent de plus en plus sélectives.

Le traitement des endroits où se reposent les glossines des groupes *morsitans* et *palpalis* a été effectué avec succès. A condition de disposer de connaissances adéquates sur la biologie des espèces en cause ainsi que sur les propriétés, dans les conditions locales, des insecticides utilisés, cette méthode s'avère efficace. Récemment les pulvérisations aériennes ont été limitées à la lutte contre les glossines de savane en Afrique orientale; au Rwanda, elles ont été utilisées avec succès à grande échelle et dans des conditions d'économie satisfaisantes. Grâce aux techniques employées au Rwanda les recherches ont permis de réduire le coût de 50%; on prévoit en outre une précision accrue de la technique qui réduira les risques de contamination du milieu par les insecticides émis par voie aérienne.

Les diverses méthodes de lutte doivent être appliquées selon les circonstances et doivent autant que possible être utilisées conjointement. Il n'existe pas de " méthode universelle ".

Les observations actuellement recueillies ne fournissent que peu de preuves quant à l'effet de la lutte chimique sur la faune; toute action de cette nature serait passagère et en tout état de cause moins marquée que celle d'autres méthodes entraînant une modification du milieu.

Le Conseil insiste sur le fait qu'aucune campagne d'assainissement, en particulier par des méthodes de lutte chimique, ne doit être entreprise isolément; elle doit faire partie d'un plan intégré de mise en valeur des terres, dont les étapes doivent être rigoureusement respectées afin de conserver le bénéfice de la campagne contre les glossines. De même, le coût des mesures anti-tsé-tsé ne sera pas examiné isolément, mais en rapport avec le coût du plan de mise en valeur. Des mesures spéciales pourront être requises pour enrayer des épidémies de trypanosomiases ou l'avance des glossines.

POINT 3. — TRYPANOSOMIASE HUMAINE

Rapporteurs: Médecin-Général-Inspecteur P. Richet
Dr R. J. Onyango
Dr H. J. C. Watson
Dr P. De Raadt

3.1. Epidémiologie

3.1.1. Information sur l'endémicité, la recrudescence ou l'apparition de nouveaux foyers

Le Professeur van den Berghe présente une communication sur le foyer de trypanosomiase à *T. rhodesiense* du Burundi. Une poussée épidémique semble se développer au Nord de Muhinga, qui est comparable au phénomène observé autour du lac Victoria.

Le Dr Burke décrit la situation de la trypanosomiase humaine en certaines régions du Congo–Léopoldville où le dépistage précoce des cas est devenu rare et où le plupart des malades présentent maintenant des troubles psychiques. La menace qui pèse sur ces régions est exprimée par les indices qu'il présente: dans certains foyers situés le long de la frontière angolaise, les indices de contamination atteignent de 3 à 6%.

Le Médecin-Général-Inspecteur Richet présente une communication qui

exprime nettement la situation satisfaisante dans les Etats francophones de l'Afrique de l'Ouest. Ce document contient aussi des informations précieuses sur la recherche des HB_2M (taux élevés de la fraction I_2M d'immunoglobulines dans le sérum) pour le diagnostic de la trypanosomiase et un compte rendu des traitements utilisés dans les différents Etats membres de l'OCCGE.

3.1.2. Facteurs d'endémicité et de propagation de la trypanosomiase humaine: réservoirs d'infection, contact homme-mouche, déplacements des personnes infectées

Il est prouvé que la trypanosomiase résiduelle, lorsqu'elle est mal ou trop irrégulièrement contrôlée, persiste et peut à tout moment donner lieu à des réveils de type épidémique local susceptibles d'essaimer en nappe et à distance.

Il est prouvé aussi que, lorsque les indices de contamination deviennent élevés, la mise en action de services — ou tout au moins d'équipes — spécialisés dans la lutte contre les grandes endémies et dotés de moyens puissants est seule capable de ramener rapidement la trypanosomiase à un taux non inquiétant pour la santé publique.

La trypanosomiase humaine à *T. gambiense* et à *T. rhodesiense* sera pourchassée par la conjugaison des moyens qui ont fait leurs preuves:

- — prospections exhaustives et minutieuses, au moins annuelles, voire semestrielles;
- — chaque fois qu'il sera possible, recherches des hyper-bêta-2 macroglobulines (HB_2M) sériques, voire du liquide de rachicentèse, qui permettra de sélectionner les sujets hautement suspects chez lesquels la recherche du trypanosome devra s'appuyer sur tous les moyens classiques de dépistages répétés autant qu'il sera nécessaire;
- — diamidinisation prophylactique des populations, sélective ou collective, selon le cas;
- — lutte contre les vecteurs par emploi d'insecticides, modification du couvert végétal ou mise en valeur des terres;
- — surveillance et contrôle des populations itinérantes.

L'accent est mis sur l'importance des prospections conjointes interterritoriales, du type de celle qui, sous l'égide de la CCTA et de l'OCCGE et avec assistance financière partielle de l'U.S. AID, est prévue dans le vieux foyer de Kissi commun à la Guinée, à la Sierra Leone et au Libéria.

3.2. Chimiothérapie

Le Dr Hawking présente une communication qui propose une hypothèse explicative sur le mécanisme de la résistance *in vitro* des trypanosomes à l'arsenic.

Le Dr Watson présente un rapport final sur les résultats du Mel.W. employé en Nigéria du Nord, indiquant que ce médicament a sa place dans la chimiothérapie de la maladie du sommeil à *T. gambiense.*

Le Médecin-Général-Inspecteur Richet expose les résultats obtenus au sein d'Etats membres de l'OCCGE qui montrent la grande efficacité du Mel.B. utilisé selon les protocoles préconisés par Neujean.

3.3. Diagnostic

Une observation des docteurs Onyango et De Raadt confirme les difficultés du diagnostic dans les périodes de basse parasitémie, la réapparition du flagellé lors des phases de haute parasitémie et les conséquences qui en découlent quant à la durée de l'observation nécessaire, en certains cas, pour mettre le protozoaire en évidence.

Le Dr Lumsden présente un exposé remarqué sur l'emploi de tests pour la détection des HB_2M et attire l'attention sur les fautes d'interprétation qui peuvent parfois en résulter. Il fait observer que la présence d'HB_2M doit être interprétée comme une présomption et non comme une certitude de trypanosomiase.

3.4. Protozoologie

Le Dr Willett décrit certains aspects de l'épidémie actuelle à *T. rhodesiense*, transmise par *G. fuscipes* dans la région d'Alego, Nyanza Central, Kenya, et émet l'hypothèse qui, tout en expliquant l'association générale entre *T. gambiense* et *G. palpalis* d'une part, et *T. rhodesiense* et *G. morsitans* d'autre part, établit qu'il n'y a pas de raison pour que l'un ou l'autre des trypanosomes ne soit pas transmis, dans des circonstances favorables, par l'un ou l'autre groupe de glossines.

Le Professeur van den Berghe exprime également certaines vues sur les rapports entre *T. gambiense* et *T. rhodesiense* et la possibilité pour ce dernier de franchir, maintenant ou dans l'avenir, la barrière écologique entre la savane et la forêt.

RECOMMANDATIONS

I. MESSAGE DE GRATITUDE

Le Conseil REMERCIE le Gouvernement de l'Ouganda de sa généreuse hospitalité. Il EXPRIME également sa gratitude au Doyen et à la Faculté de Médecine de Makerere qui a mis à sa disposition les locaux et les moyens matériels nécessaires pour la réunion. Il REMERCIE également le Commissaire des Services Vétérinaires de l'Ouganda et ses collaborateurs pour l'aide apportée à l'organisation de la réunion.

II. NOUVELLE APPELLATION DU CSIRT

Le Comité RECOMMANDE que son appellation soit, à l'avenir: Conseil Scientifique International de Recherches sur les Trypanosomiases.

III. RAPPORTS ENTRE LE CSIRT ET LES AUTRES ORGANISATIONS

Le Conseil ESTIME que ses activités seraient mieux servies par le maintien de son existence indépendante. Il devra poursuivre son association avec la CCTA et par la suite avec les Commissions appropriées de l'OUA; il devrait également collaborer étroitement avec l'OMS et la FAO.

IV. EXTENSION DES ATTRIBUTIONS DU CSIRT

Le Conseil ENTERINE la Recommandation VI de la Réunion d'Experts tenue à Lagos en septembre 1963 selon laquelle tout doit être mis en œuvre pour permettre aux chercheurs en Afrique de se tenir au courant des travaux menés ailleurs, dans le

domaine des trypanosomiases. En outre, il y aura lieu de multiplier les contacts entre chercheurs tant en matière de trypanosomiases et de leurs vecteurs qu'en d'autres domaines présentant un intérêt pour la recherche.

Par ailleurs, il est évidemment souhaitable que toutes les organisations internationales s'occupant des trypanosomiases soient pleinement associées aux travaux du CSIRT.

Le Conseil RECOMMANDE en conséquence que ses statuts soient ceux de l'ancien Comité, avec les extensions suivantes:

1) Toute organisation internationale travaillant dans le domaine de la trypanosomiase sera invitée de plein droit à désigner un représentant au sein du Conseil.
2) Les Directeurs des Instituts établis en Afrique et poursuivant un programme de recherches sur la trypanosomiase, ou leur représentant, seront également habilités à participer aux travaux du Conseil, sur invitation du Comité Exécutif.
3) A l'occasion de ses débats scientifiques le Conseil pourra coopter des personnalités venant d'autres pays où se poursuivent des recherches sur la trypanosomiase ou les problèmes connexes.

V. LE COMITE EXECUTIF

Le Conseil RECOMMANDE l'institution d'un Comité Exécutif, composé comme suit:

1) Le Président du CSIRT.
2) Quatre autres experts reconnus, choisis par le CSIRT et représentant un éventail de disciplines aussi étendu que possible.
3) Un représentant de l'OMS, un représentant de la FAO et, sur invitation du CSIRT, un représentant de tout autre organisme apportant une contribution financière substantielle.

Le Comité aura, entre autres, les attributions suivantes:

1) Agir en tant que Comité Directeur du CSIRT.
2) Convoquer des réunions *ad hoc* de spécialistes en cas de besoin.
3) Agir en qualité d'organisme consultatif sur:
   a) les priorités dans les programmes de recherche et de lutte;
   b) l'appui à apporter aux recherches sur les trypanosomiases;
   c) la répartition des fonds;
   d) les recherches à entreprendre.
4) Rendre compte au Conseil de ses activités à intervalles réguliers.

Le Comité se réunira annuellement ou plus fréquemment s'il y a lieu. A cet effet, les prévisions budgétaires devront être établies, en premier lieu, en vue de deux réunions par an.

Le Conseil ESTIME souhaitable que les réunions du Comité se tiennent à tour de rôle dans les diverses régions d'Afrique où sévissent les trypanosomiases.

VI. PROJET DE CREATION D'UN BUREAU AFRICAIN DE LA TRYPANOSOMIASE

Le Conseil CONSIDERE qu'en raison de l'institution de son Comité Exécutif ainsi que la création par l'OMS d'un service d'informations sur la trypanosomiase, la décision portant création d'un Bureau Africain de la trypanosomiase doit être différée jusqu'à ce que la nécessité d'un tel bureau soit clairement établie.

VII. RAPPORT DU CONSEIL

Le Conseil ESTIME que les rapports complets de ses réunions, y compris les communications scientifiques et les résumés des discussions, présentent le plus haut intérêt pour les chercheurs dans le domaine de la trypanosomiase. Il RECOMMANDE que le rapport de la présente réunion soit publié dès que possible.

Il SOUHAITE qu'il soit possible de distribuer des tirés à part aux auteurs de communications scientifiques.

VIII. UNIFORMISATION DE LA PRESENTATION DES COUTS D'UNE CAMPAGNE

Le Conseil RECOMMANDE l'uniformisation, sous la forme ci-dessous, de la présentation des coûts d'une campagne de lutte chimique anti-glossines:

A. Moyens

— Véhicules et remorques (type et prix).
— Matériel de pulvérisation (type et prix).
— Installations (matériel de campement, dépôts, piste d'atterrissage, atelier roulant, etc.).
— Tout autre matériel.

B. Couts

	Travaux préliminaires et de controle Quantité/Cout	*Exécution* Quantité/Cout	*Frais d'entretien* Quantité/Cout
I. Personnel			
a) Scientifique			
b) d'Exécution			
c) Transport du personnel			
d) Frais d'administration			
II. i) Opérations de pulvérisation			
a) Matériel de pulvérisation			
b) Insecticides			
c) Diluants			
d) Divers			
ii) Frais de mise en place			
a) Matériel de pulvérisation			
b) Insecticides			
c) Diluants			
d) Divers			

III. **Opérations annexes**

(Isolement, barrières, etc.)

IV. **Installations**

(Bâtiments, matériel de campement, dépôts, pistes d'atterrissage, ateliers, pistes d'accès, etc.)

V. Nombre de km traités ou surface traitée. Prix de revient au km ou à l'ha.

C. Estimation de la valeur du matériel recupérable

Définition des termes employés

Travaux préliminaires et de contrôle

Doivent être compris sous cette dénomination:

— les travaux d'investigation préliminaire
— les photographies aériennes et la cartographie
— la reconnaissance du terrain
— la mise en place des contrôles biologiques
— la coordination administrative
— le planning des opérations
— la commande et la supervision de l'acheminement du matériel et l'approvisionnement.

Frais d'entretien

Coût de toutes mesures propres à maintenir et à consolider les résultats de l'opération.

Les **coûts** seront exprimés de la façon suivante:

I. *a*) somme globale;
b) pour le personnel sous contrat: prix de revient mensuel
pour la main-d'œuvre: hommes-jours;
c) coût total.

II. Coût effectif du matériel, accessoires, pièces de rechange, entretien.

Il y a lieu d'établir une nette distinction entre:

a) l'aire de l'habitat permanent;
b) l'aire de dispersion maxima.

Ces deux aires s'entendent libérées de l'infestation par les glossines.

SECTION I

ANIMAL TRYPANOSOMIASIS/TRYPANOSOMIASES ANIMALES

SOME OBSERVATIONS ON THE TREATMENT OF CATTLE WITH BERENIL

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with technical assistance of

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Since Berenil, 4·4′-diamidinodiazoaminobenzene, was put into use in 1955, it has been generally accepted that this drug is rapidly excreted from treated animals (Bauer, 1959; MacOwan, 1961; Annual Report, Veterinary Department Kenya, 1960; Fairclough, 1962 and Hutchinson and Watson, 1962). Fairclough (1963), however, came to the conclusion " that Berenil at 7 mg./kg. remained in the adult bovine animal long enough to protect it from reinfection for at least twenty-four hours ".

Two field observations on Berenil in cattle at Lugala, Uganda, an area where the trypanosome risk is high, led us to believe that it might persist in the animal for considerably longer than twenty-four hours, and the phenomenon was further investigated by a laboratory and a field experiment.

FIELD OBSERVATIONS

(1) Twenty cattle were sent to Lugala, to investigate the immune response of cattle exposed continuously to natural trypanosome infection and treated at regular intervals with Berenil intramuscularly at 7 mg./kg. They were treated at weekly intervals for the first three weeks and thereafter fortnightly. Weekly examinations were made for the presence of trypanosomes, by inoculation of blood into mice and by examination of thick blood films stained with Giemsa.

On day seven, fifteen out of the twenty cattle were found positive. All twenty cattle received their first Berenil treatment on this day and only a further two were subsequently found positive up to day sixty-two, when Berenil treatment was stopped. All twenty were negative on being examined on day sixty-two and were left in the area, without treatment, for a further twenty-eight days. At the end of this period all were positive. From the last Berenil treatment two animals took fourteen days to become positive, thirteen took twenty-one days and five took twenty-eight days.

On day sixty-two a further four cattle, which had not previously been exposed to trypanosome infection, were sent to Lugala. Two of these had undergone the same Berenil regime as the above twenty cattle and two were untreated. The two treated animals took between twenty-one and twenty-eight days to become positive, whereas the two untreated were both positive on the seventh day after exposure to infection.

(2) During 1964 three cattle have been used regularly at Lugala in conjunction with catches of tsetse. At first, these animals remained in the area for five days each week and were then brought back to the laboratory where blood was inoculated

into mice, thick blood films prepared and they were then treated with Berenil intramuscularly at 7 mg./kg. After the experience gained from the first observation, it was decided to treat them instead at fortnightly intervals with Berenil at 7 mg./kg.

None of these three animals has ever shown a parasitaemia (J. M. B. Harley personal communication).

FIELD EXPERIMENT

Ten cattle were treated with Berenil 7 mg./kg. intramuscularly and sent to Lugala, together with ten untreated animals. They remained at Lugala for eight days, and were returned to EATRO on the ninth day.

On day ten, all ten untreated and one treated animal were positive. Of the nine remaining treated cattle seven animals became positive on day thirty-one and two on day fifty-seven by thick blood film examination carried out daily, except Sundays.

LABORATORY EXPERIMENT

Defibrinated blood was collected from four cattle prior to inoculation with Berenil 7 mg./kg. intramuscularly. After one hour, and then seven, ten, fourteen and twenty-one days after inoculation, defibrinated blood was collected. The blood was centrifuged and each of the resulting sera used as diluent for titration in mice of a Berenil-sensitive *Trypanosoma brucei* Subgroup isolate (EATRO-588) using the method described by Lumsden *et al.* (1963). By comparing the infectivity of these trypanosomes after exposure *in vitro* for four hours at 27° C. in each of these serum samples, an estimate can be made of the degree of infectivity inhibited per unit volume of serum.

The results of this experiment are shown in the following table where the figures represent the number of $\log_{10}$ ID_{63}'s inhibited by 1 ml. of serum.

Animal number	Time after Berenil inoculation				
	1 hour	7 days	10 days	14 days	21 days
S 1 . . .	⩾6·0	3·8	2·5	1·8	0·8
S 81 . . .	⩾6·0	3·5	3·0	1·5	1·6
S 75 . . .	⩾6·0	3·2	3·4	2·5	1·0
S 47 . . .	⩾6·0	3·0	2·8	2·2	1·0

From this table it can be seen that one hour after the inoculation of Berenil there was a high degree of inhibition of trypanosome infectivity as judged by this method. As differences of less than 1·2 log ID_{63}'s are insignificant (Lumsden *et al.*, 1963) it will be seen that by day twenty-one the inhibition of infectivity was insignificant in three out of the four cattle, but in the fourth it was still significant.

CONCLUSION

From these experiments and observations we conclude that Berenil inoculated at 7 mg./kg. intramuscularly is not rapidly excreted.

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ESSAIS DES MEDICAMENTS TRYPANOPREVENTIFS CHEZ LES ANES

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Les médicaments trypanopréventifs ont été surtout essayés et utilisés chez les bovins et à un degré moindre chez les porcs. Pour les autres espèces, fort peu de choses ont été publiées tant sur la toxicité que sur l'efficacité de ces traitements; le CSIRT de Jos en 1960 a d'ailleurs attiré l'attention sur cette lacune et demandé que des recherches soient entreprises sur ce sujet.

L'élevage des ânes dans les zones à glossines en vue de leur utilisation pour les travaux agricoles et les petits transports, présenterait un intérêt certain; il nous a donc paru intéressant d'étudier les possibilités d'emploi des médicaments trypanopréventifs, chez ces animaux.

Les essais qui ont été publiés sont très rares et ne portent que sur quelques animaux (Davey, Pellegrini et Bonelli). La plupart des expériences ont été faites sur les chevaux et le plus souvent les trypanocides étaient utilisés dans un but curatif: le bromure de dimidium, l'Ethidium sont généralement bien tolérés par les chevaux, mais ces deux médicaments n'ont qu'une très faible valeur préventive. L'Antrycide est très mal supporté, et il provoque souvent la formation d'abcès importants. De plus sa toxicité générale n'est pas négligeable. Le Bérénil n'est pratiquement pas toxique mais son action est strictement curative. Le chlorhydrate de chlorure de métamidium (M & B 4404) paraît plus intéressant: Bouchard et Dick ont montré que ce médicament pouvait être utilisé chez les chevaux, en injection intramusculaire à l'encolure, à la dose de 2 mg par kg.

A. — TECHNIQUE D'ETUDE

Nous avons essayé les trypanocides, qui dans les autres espèces et particulièrement chez les bovins, donnent les durées de protection les plus longues.

Seize ânes, provenant du Tchad, ont été répartis en 4 lots et traités ainsi:

1er lot: Prothidium . . . . . 2,5 mg par kg
2e lot: Chlorhydrate de chlorure d'isométamidium = Samorin . . 2 mg par kg
3e lot: M & B 4596 . . . . . 2 mg par kg
4e lot: Témoins non traités

Tous les traitements ont été faits par voie intramusculaire à l'encolure.

Les ânes ont ensuite été placés à la station de Béwiti où ils sont soumis aux attaques de *Glossina fusca* et *G. fuscipleuris*, le trypanosome le plus fréquent était *T. congolense* (on doit noter que *T. brucei* n'a jamais été trouvé à la station de Béwiti).

Deux fois par semaine des gouttes épaisses sont faites sur tous les animaux.

B. — RESULTATS

1. Toxicité

Réactions locales

Tous les animaux traités présentaient, dans les jours suivant le traitement, des réactions locales importantes, atteignant un diamètre d'une vingtaine de centimètres.

Aucun abcès ne se produisit et les réactions rétrocédèrent au bout d'une quinzaine de jours.

Deux mois après le traitement les réactions étaient à peine perceptibles et trois mois après elles étaient totalement disparues.

Toxicité générale

Aucun accident toxique n'a été observé et tous les traitements ont été bien supportés.

2. Propriétes préventives

Malgré les conditions expérimentales très dures qui règnent à la station de Béwiti, où les bovins non traités s'infectent régulièrement en moins d'un mois, les ânes ont fait preuve d'une résistance remarquable aux trypanosomiases:

Témoins

Chez les ânes non traités les trypanosomes ne sont apparus qu'après 43, 63, 98 et 123 jours.

Anes traités au Prothidium

Pour deux animaux la protection a été de 265 et 275 jours. Les deux autres ânes sont encore indemnes 280 jours après le traitement. Dans les mêmes conditions, la durée de protection varie, chez les bovins, entre 4 et 6 mois.

Anes traités au Samorin

Deux animaux sont morts accidentellement. Chez les deux autres les trypanosomes sont apparus 233 et 242 jours après le traitement. Chez les bovins, placés dans des conditions identiques, la protection ne dépasse pas trois mois.

Anes traités au M et B 4596

Deux animaux sont encore indemnes 280 jours après le traitement. Pour les deux autres la durée de protection a été de 143 et 175 jours. Pour les bovins elle est de l'ordre de 5 mois.

Dans tous les cas le trypanosome en cause a été *T. congolense* mais on peut penser que si *T. brucei* avait été présent à la station de Béwiti, les résultats auraient été très différents, les médicaments que nous avons utilisés n'ayant qu'une faible action sur ce trypanosome.

Tous les animaux infectés ont été traités au Bérénil, à la dose de 3,5 mg par kg, par voie sous-cutanée; ce traitement a été bien supporté et a toujours été efficace, sauf pour un animal chez qui un deuxième traitement à la dose de 7 mg par kg a été nécessaire pour obtenir la guérison.

CONCLUSIONS

Les ânes non traités se sont montrés relativement peu sensibles aux trypanosomiases et n'ont été trouvés infectés qu'après des délais bien supérieurs à ceux qu'on observe chez les bovins placés dans les mêmes conditions.

— Les trois trypanocides qui ont été utilisés: Prothidium, Samorin et M & B 4596 sont assez bien tolérés par les ânes, les réactions locales qu'ils provoquent n'étant que passagère et sans conséquence grave.

— La durée de protection conférée par ces trois médicaments est notablement supérieure à celle qu'on note chez les bovins.

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RAPPORT SUR LES PROGRES RECENTS DE LA CHIMIOTHERAPIE DES TRYPANOSOMIASES ANIMALES

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Il faut bien reconnaître que depuis la dernière réunion du CSIRT à Conakry en 1962, la chimiothérapie des trypanosomiases animales n'a pas sensiblement progressé: aucun médicament nouveau n'a été découvert et les travaux qui ont été publiés pendant ces deux dernières années, ont surtout porté sur l'utilisation de produits déjà bien connus, qui appartiennent aux trois groupes chimiques classiques:

— les phenanthridines
— les diamidines
— les quinaldines.

A. —LES PHENANTHRIDINES

I. Composés nouveaux

Les recherches sur l'action trypanocides des phenanthridines ont été poursuivies par Berg et son équipe. Elles ont permis de préciser nos connaissances sur les rapports entre la constitution chimique et l'activité trypanocide, mais elles ne semblent pas avoir aboutit à la mise au point de produits intéressants d'un point de vue pratique.

II. Métamidium et Isométamidium

Le chlorhydrate de chlorure d'isométamidium qui est maintenant commercialisé sous le nom de Samorin [1] a été essayé dans différents pays.

1. Essais sur les bovins

a) Propriétés curatives

Kirkby, au Nigéria, a confirmé l'intensité de l'action curative de l'isométamidium qui agit à des doses aussi faible que 0,1 mg par kg; on doit noter cependant que dans cette expérience le trypanosome en cause était *T. vivax*, et que ce trypanosome est de beaucoup le plus sensible aux phenanthridines: d'ailleurs, dans les mêmes conditions, l'homidium agissait à la dose de 0,25 mg par kg soit le quart de la dose habituelle.

Finelle, en République Centrafricaine, a également obtenu d'excellents résultats, aussi bien sur *T. vivax* que sur *T. congolense.*

[1] May & Baker Ltd.

Fairclough, au Kénya, a essayé le métamidium, puis l'isométamidium à la dose de 0,5 mg par kg, le bétail n'étant traité qu'au fur et à mesure que les trypanosomes apparaissaient dans le sang. Dans ces conditions les traitements ont été espacés en moyenne de:

— 29 semaines pour le métamidium
— 14,2 semaines pour l'isométamidium
— 8 semaines pour le Bérénil
— 8,9 semaines pour l'Ethidium.

b) **Propriétés préventives**

L'action préventive du métamidium et de l'isométamidium a été testée par plusieurs auteurs.

Médicaments	Dose	Protection moyenne	Extrêmes	Auteurs
Métamidium	2	226	23–398	Robson
	3	132,6	109–191	Gray et Stephen
	4,75	192	131–243	Gray et Stephen
Isométamidium	0,5	62	47–76	Finelle
	1	83	59–109	Finelle
	2	90	59–121	Finelle
	2	258	93–398	Robson
	4	351	304–401	Robson

L'isométamidium a donc une action préventive très nette mais tous les auteurs reconnaissent la variabilité de son action et la facilité avec laquelle apparaissent des trypanosomes résistants.

c) **Toxicité**

Toxicité générale. — L'isométamidium est bien supporté aux doses habituellement utilisées de 1 et 2 mg par kg. Par contre il est toxique à la dose de 4 mg par kg (Robson).

Toxicité locale. — L'injection intramusculaire de l'isométamidium provoque des réactions locales, parfois importantes, qui regressent en deux à trois mois, mais qui ne semblent pas influer fâcheusement sur l'état général (Robson — Finelle). Par contre l'injection sous-cutanée provoque des réactions très violentes qui condamnent ce mode d'emploi (Gray et Stephen).

L'injection intraveineuse est bien tolérée au moins chez les bovins (Finelle).

2. Essais sur les chevaux

Bouchard et Dick (1962) ont essayé le métamidium, dans le traitement de la trypanosomiase du cheval à *T. vivax*. A la dose de 2 mg par kg, par injection intramusculaire à l'encolure, ce médicament est utilisable, à condition de prendre la précaution de fractionner les doses. Il est par contre très toxique par voie intraveineuse.

III. M & B 4596

Ce nouveau trypanocide, apparenté à l'isométamidium, n'a été l'objet que de peu de travaux. Il présente pourtant un intérêt certain et son expérimentation

mériterait d'être poursuivie, puisque dans les conditions très dures où il a été essayé, il a conféré une protection atteignant 5 mois (Finelle). Il provoque cependant, comme les autres médicaments appartenant au même groupe, des réactions locales, très variables en importance mais paraissant n'avoir pas de conséquences fâcheuses sur l'état général des animaux.

IV. Complexe Suramine-Prothidium

Stephen dans une expérience réalisée au Nigéria, a montré que le couplage du Prothidium [2] et de la Suramine entraînait une nette augmentation de l'activité préventive, et une diminution de la toxicité générale; malheureusement les réactions locales restent très importantes et l'utilisation pratique de ce produit ne peut être envisagée.

B. — LES DIAMIDINES

Bérénil

Les essais réalisés avec le Bérénil [3] tant au laboratoire que sur le terrain ont confirmé les qualités exceptionnelles de ce trypanocide (Fairclough, Sobrero). Sa toxicité très faible (on peut impunément injecter des doses six fois supérieures à la dose habituelle), son action sur les trypanosomes résistants aux autres thérapeutiques, sa très faible aptitude à créer lui-même des souches résistantes, en font le traitement de choix des infections à *T. congolense* et *T. vivax*.

L'élimination du Bérénil a été étudiée par Cunningham et collaborateurs, qui ont montré que sa vitesse d'élimination peut être beaucoup moins rapide qu'on ne le pensait jusqu'à maintenant, et que 21 jours après l'injection intramusculaire d'une dose de 7 mg par kg, le sérum présentait encore une activité trypanocide.

Fairclough a prouvé que le Bérénil pouvait permettre le maintien du bétail dans les régions contaminées:

— Si le risque d'infection est faible, on peut se contenter de traiter les animaux malades au fur et à mesure que les cas de trypanosomiase apparaissent.

— Si le risque est plus élevé, on doit traiter systématiquement tous les deux mois l'ensemble du troupeau. En un an Fairclough a ainsi pu faire tomber le taux d'infection de 28 à 2%'

C. — LES QUINALDINES

Quinapyramine (Antrycide) [4]

T. simiae

Stephen, Soares Machado ont montré l'intérêt du chlorure d'Antrycide et du complexe Antrycide-Suramine tant pour le traitement que pour la prévention de la trypanosomiase porcine à *T. simiae*, trypanosome sur lequel l'Antrycide s'avère être le seul trypanocide vraiment efficace. Il est cependant nécessaire d'injecter des doses considérables (respectivement 50 et 40 mg par kg) et on peut se demander si le prix de revient de ce traitement n'est pas prohibitif.

[2] Boots Pure Drug Co.
[3] Farbwerk Hoechst AG.
[4] Imperial Chemical (Pharmaceutical) Industries Ltd.

T. evansi

Sur des animaux de laboratoire le complexe Suramine-Antrycide a donné de bons résultats dans le traitement et la prévention de la trypanosomiase à *T. evansi* (Gill et Malhotra, Gill) et il serait intéressant que ces essais soient poursuivis sur les grands animaux.

D. — ASSOCIATIONS

Robson a essayé d'injecter simultanément deux médicaments trypanopréventifs, chacun d'eux étant utilisé à une dose inférieure à la dose habituellement employée.

Médicaments	Doses	Protection moyenne	Extrêmes
Prothidium + Isométamidium	0,5 + 0,5	164	111–196
Prothidium + Prosalt	0,5 + 1,85	164	93–209
Isométamidium + Prosalt	0,5 + 1,85	156	37–195
Prosalt	7,4	124	84–195

Cette technique paraît intéressante puisqu'elle semble permettre, en réduisant les doses injectées:

— d'abaisser le prix de revient des traitements,

— de diminuer les réactions locales que provoquent la plupart des trypanopréventifs employés à la dose habituelle.

Elle mériterait de nouvelles études.

E. — MODE D'ACTION

Le mode d'action des trypanocides est encore très mal connu, bien que plusieurs travaux récents aient apporté des précisions.

1. Fixation des trypanocides dans les tissus

Benazet, Hill et McFadzean ont recherché ce que devenaient les trypanopréventifs après leur injection. Ils ont montré qu'alors qu'une partie de ces médicaments se déposait au niveau du lieu d'injection, provoquant une réaction locale plus ou moins importante, une autre partie va se fixer dans certains organes, en particulier le foie et le rein. Mais pour Benazet ce serait cette fixation tissulaire qui conditionnerait en grande partie l'activité préventive, alors que Hill et McFadzean pensent qu'au contraire c'est le dépôt qui se fixe au lieu d'injection qui joue le rôle essentiel.

2. Action des trypanocides *in vitro*

La Tryparsamide et le Bérénil agissent *in vitro*, et détruisent les cultures de *T. rhodesiense* et *T. congolense*, à la concentration de 1 pour 10.000, alors que,

dans les mêmes conditions, l'Antrycide, l'homidium, le Prothidium, l'isométamidium sont inactifs (Hawking).

3. Action des trypanocides sur les trypanosomes des glossines

Hawking a étudié l'action que pouvaient avoir les trypanocides sur les trypanosomes des glossines: à la concentration de 1/10.000 l'Antrycide, l'isométamidium, le Bérénil détruisent les trypanosomes, le Prothidium n'est actif que sur *T. vivax*, l'homidium est inactif a cette concentration et à 3/10.000 il est toxique pour les glossines.

4. Mécanismes de l'action trypanocide

Ormerod et Shaw ont recherché, au microscope à contraste de phase ou à fluorescence, les modifications produites par les trypanocides, dans la morphologie des trypanosomes, et ont fait l'étude des granulations qui apparaissent ou se multiplient sous l'influence de ces médicaments.

5. Action carcinostatique des phenanthridines

Au risque de déborder du sujet, nous ne voulons pas passer sous silence le travail de Kandaswamy et Franck Henderson, qui ont montré que l'Ethidium avait une action carcinostatique. Ce produit bloquerait en effet la synthèse des acides nucléiques en inhibant l'incorporation des bases puriques.

Ce travail présente peut-être un intérêt en ce qui concerne la thérapeutique du cancer, mais en ce qui nous concerne, il doit également contribuer à élucider le mode d'action des trypanocides: de toute façon, cette question mérite de plus amples recherches.

F. — CHIMIORESISTANCE

1. Apparition de la chimiorésistance

Le Bérénil est connu pour être pratiquement inapte à créer des trypanosomes résistants: Whitside a essayé de rendre résistante une souche de *T. congolense* par la technique habituelle des sous-dosages; cela nécessita des traitements beaucoup plus nombreux que pour les autres trypanocides et le taux de résistance ne fut jamais très élévé: 4 mg par kg alors que la souche initiale réagissait à 1 mg par kg.

Travaillant sur l'homidium, Stephen n'a pu rendre résistante une souche de *T. vivax* transmise par les glossines.

Hawking a montré que chez les animaux de laboratoire, la résistance à l'Antrycide apparaît plus rapidement que celle au Prothidium ou au métamidium, mais elle disparaît également plus rapidement.

2. Résistance croisée

Pour Hawking la résistance des trypanosomes à l'Antrycide, à l'homidium ou au Bérénil dépendent de récepteurs distincts et varient donc séparément. Le Prothidium et le métamidium se rattachent au groupe des phénanthridines, en dépit de leur parenté respective avec l'Antrycide ou le Bérénil. L'Antrycide par contre provoque des résistances croisées avec les phénanthridines et parfois une légère résistance au Bérénil.

Cette résistance au Bérénil peut donc être de trois types:

— une résistance croisée avec l'Antrycide, qui est toujours légère,

— une résistance environ 5 fois supérieure, due au Bérénil lui-même,

— une résistance plus de 100 fois supérieure provoquée par la Stilbamine.

3. Virulence

Stephen et Hill ont noté que les trypanosomes réapparaissant à la fin de la période de protection consécutive au traitement par les trypanopréventifs, trypanosomes qui sont souvent résistants, avaient une pathogénicité très réduits; les raisons de ce phénomène, qui a déjà été signalé à plusieurs reprises, est inconnue, mais on peut penser qu'il s'agit d'un mécanisme de nature immunitaire, qui nécessiterait de nouvelles recherches.

G. — TRAVAUX D'ENSEMBLE

Pour terminer, il faut signaler plusieurs travaux d'ensemble. Ce sont d'abord les mises au point détaillées et très complètes de Williamson et de Walls, qui exposent l'évolution de la chimiothérapie des trypanosomiases et font le point des connaissances actuelles. C'est ensuite le travail de Stephen, sur la trypanosomiase porcine à *T. simiae* et celui de Whiteside, sur l'aspect économique de l'utilisation des trypanocides.

On doit également signaler le rapport du comité d'experts sur les trypanosomiases, qui, bien que réuni par l'OMS, s'est intéressé aux problèmes posés par les trypanosomiases animales. Nous ne pouvons d'ailleurs mieux faire que de conclure avec lui que:

Malgré les grandes améliorations récémment apportées au traitement de la trypanosomiase du bétail, des progrès restent à faire sur certains points. Il faudrait en particulier découvrir:

a) **des médicaments prophylactiques** conférant une protection plus prolongée, utilisables dans les régions fortement exposées et ne présentant pas les inconvénients de la résistance croisée;

b) **de nouveaux groupes de médicaments curatifs** pour donner un plus grand choix de produits ne provoquant pas de résistance croisée;

c) en outre la mise au point de médicaments curatifs et prophylactiques contre les infections à *T. simiae* du porc serait d'une grande utilité.

Tous les médicaments trypanocides actuels ont été découverts empiriquement. Il est donc nécessaire d'entreprendre des études fondamentales sur la **physiologie** et le **métabolisme** des trypanosomes ainsi que sur **l'action biochimique** des médicaments; elles pourront aboutir à la mise au point rationnelle d'agents chimiothérapiques agissant suivant des mécanismes connus.

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PROPHYLAXIS AND SUPPRESSION OF TRYPANOSOMES BY CHEMOTHERAPEUTIC COMPOUNDS WITH REFERENCE TO DRUG RESISTANCE AND REGIMES

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One of the great dangers of chemotherapeutic control of bovine trypanosomiasis by regular treatment at stipulated time intervals is the possible appearance of drug resistance in the trypanosomes. A considerable amount of work has been done on this in the last few years, particularly by Whiteside (1960) and Fairclough (1962). It is not the purpose of this communication to discuss drug resistance as such, but to present a possible reason for its development in the field usage of chemotherapeutics and its prevention.

For the purposes of this paper it is necessary that three terms must be defined. Prophylaxis is already defined as the prevention of disease, but this is insufficiently precise. It is proposed, therefore, to introduce the term **trypanocidal prophylaxis** to indicate the **prevention of infection** and the term **suppression** to indicate the **prevention of patent infection.** The third term is the **protection period** which indicates the time to patent infection.

It has been shown by Robson and Milne (1957) that Prothidium can suppress a known infection up to fifty days. This means that the prepatent period is extended from 7–12 days to 15–50 days. The protection period of Prothidium is approximately 3–6 months depending on the trypanosome risk (numerous works). Van Hoeve and Cunningham (this Conference) have also demonstrated that Berenil is capable of suppressing patent infection up to a minimum of forty-nine days. On average the extension of the prepatent period with Berenil appears to be approximately three weeks. Other data from this paper show the protection period for Berenil at 7·0 mg./kg. is up to three to four weeks.

From these results it would appear that there are two distinct periods in the protection period noted for a drug during a field trial. The first is the time when a trypanocidal prophylaxis prevails; the second when the infection is merely suppressed. With Prothidium and possibly other prophylactics this period of trypanocidal prophylaxis may last for some weeks. Practically speaking it would be difficult and expensive to demonstrate and prove adequately the length of each of these periods for each drug. Fairclough (1962) has infected cattle, twenty-four hours after treatment with Berenil at 3·5 mg./kg., with trypanosomes. Cawdery and Simmons (this Conference) have shown that doubling the dosage rate of drugs (Samorin 0·5 mg./kg. and 1·0 mg./kg.; Prothidium 1·0 mg./kg. and 2·0 mg./kg. and others) had little apparent effect on the protection period. Other workers (Smith, 1959 and Lyttle, 1960) have also noted this effect. Robson and Cawdery (1958) however showed a difference between 2·0 mg./kg. and 4·0 mg./kg. of Prothidium, as did Lyttle (1960), using grade cattle. In view of this work it is suggested that the trypanocidal prophylaxis afforded by Berenil irrespective of dose is likely to be very short, though it is admittedly impossible to make a precise assessment of the duration of the trypanocidal prophylaxis.

This work noted above is important because it brings out evidence which supports the concept of the suppression period, and a trypanocidal prophylactic period in relation to the protective period noted in field drug trials. The period of suppressed infection is of considerable importance because during this time the trypanosomes are exposed to non-fatal concentrations of the trypanocide, which is, basically, the classical method of producing drug resistance. It is therefore of some importance to appreciate this fact when interpreting the results of a field trial and determining a satisfactory regime for trypanoprophylactics.

To understand the theoretical basis of this suppression period the absorption and excretion pattern of the drugs and their chemical characteristics must be understood. Unfortunately too little work has been done on this aspect of trypanosome chemotherapy. Spinks (1950) has done some work on Antrycide (quinapyramine—B. Vet. C.) showing the rapid absorption and excretion from the circulation, and the formation of secondary depots in liver and kidney. Taylor (1960) has shown that Prothidium forms depots in the tissues at the site of injection and in the liver. The kidneys show less absorption. Unfortunately both techniques were not sensitive enough to estimate the drug content of fluids and tissues for a satisfactory period. Cawdery (unpublished data) has noted staining of the injection site for up to two months with Prothidium and up to four months with Samorin. From van Hoeve and Cunningham work (this Conference) it is apparent that something happens with Berenil though to a lesser degree.

The theoretical explanation of this rapid absorption and excretion from the circulatory system and the creation of secondary depots in the tissues is possibly most easily explained by the nature of the drugs themselves. They are all strongly basic and the phenanthridines (Knight and Cawdery, unpublished data) show a strong affinity for nucleic acid. In fact, they may be precipitated out of solution as a " nucleate ". Taylor (1960) indicates that Prothidium may be associated with proteins. They also show a strong affinity for certain bacteria and tissue cells. As the other drugs are somewhat similar it is reasonable to suppose that they would show the same affinities though in different degrees. The rapid excretion noted from the circulation is then probably due in part to simple chemical excretion, in part to metabolism and in part to the absorption of the tissue cells, particularly liver and kidneys; this latter possibly in association with the various nucleic acids of the cells, proteins and other cellular elements. The periods of sterile prophylaxis and suppression are presumably dependent on the release of the drug from the tissues. This release may possibly be somewhat similar to the decay of a radioisotope, the rate being governed by the affinity of the drug for cellular components, its breakdown rate and physical excretion. This concept is partially borne out by van Hoeve and Cunningham (this Conference). The times seven days, ten days, fourteen days and twenty-one days are roughly logarithmic and the average drops in $\log_{10} ID_{63}$ between these days are 0·45, 0·9 and 0·9; there is an indication, no more, that a logarithmic downward trend exists. It would be of considerable value if this work could be repeated in greater detail in association with a single day's exposure to tsetse at various days after treatment.

If this concept is correct, then the demonstration of drugs in the tissues and fluids of the body could be expected even after the loss of demonstrable biological effect, providing a sufficiently sensitive method of analysis is available. Furthermore, it could explain the apparent lack of effect of increasing dosage rate in that the lower dosage rates provide an adequate concentration to largely saturate the

absorption capacity of the main tissues involved. Further increases under these circumstances would not appreciably alter the absolute amount of drug absorbed unless they were of considerable magnitude. Taylor (1960) used two dosage rates of Prothidium, but different routes of injection. However, both dosage rates (3·7 mg./kg. intracardially and 7·5 mg./kg. intraperitoneally) gave similar liver concentrations. Unfortunately, little work has been done on tissue concentrations after different dosage rates so that this supposition is based largely on the practical field results noted above.

This concept might also account for the apparent absence of a preferential route of administration (Cawdery and Simmons, this Conference). Furthermore, the hoped-for improvement of the protective period by the use of high-viscosity vehicles for the administration of drugs which did not materialise may also be accounted for by the fact that the day-to-day release of drug to an effective state was not changed, even though the actual source, i.e. a tissue depot or a mechanical depot, was so changed.

Under these circumstances it would appear that the present regimes used for chemoprophylaxis of trypanosomes include necessarily a potential danger of drug resistance in the trypanosomes. The fact that drug regimes have proven satisfactory in many cases indicates that the primary levels attained in the first twenty-four hours after injection, or the levels attained during the period of trypanocidal prophylaxis, are sufficiently high to cure the suppressed infection where it exists. The latter possibility is considered the less likely one. These primary levels are likely to be closely associated with the dosage rate. This, in fact, has been shown to be the case with Antrycide methylsulphate by Spinks (1950) though not with the chloride due to its insolubility. While no marked difference between X mg./kg. and 2X mg./kg. is noted in the protective period in a single dose comparative trial where the cattle start free of trypanosomes, it is quite possible that 2X mg./kg. is necessary on the second and subsequent injections when the cattle have a suppressed infection; the extra drug being required to establish the necessary primary curative levels, the protective period not being affected.

If this is the case, determination of the regime must be very carefully considered. There are three (or more) alternatives which might well be examined with advantage.

The first alternative is the use of the prophylactic at a sufficiently high dosage rate to ensure complete eradication of any suppressed infection. This is probably the method used in most control work. The main disadvantage of this method may be that too low a dose is used, thus allowing the slow development of drug resistance. The second alternative is to use the prophylactic at a lower level with the incorporation of a sanative treatment at regular intervals within the framework of the regime; for example, before every fourth treatment with the prophylactic drug. The third alternative is to use the prophylactic at the minimum level required to attain the necessary tissue levels capable of maintaining a reasonable protective period. This alternative would need the incorporation of a sanative treatment with each prophylactic treatment to obtain radical cure. This may be the alternative of choice as a method of preventing the development of drug resistance, particularly if the period of **trypanocidal prophylaxis** of the prophylactic drug covers the period of **suppression** of the sanative. It is possible that with this alternative the high-viscosity base would work best by allowing a greater reduction of the chemoprophylactic than the aqueous solutions by reducing the

primary high levels of drug. It is possible that with these bases the primary levels of drug are inadequate for curative purposes. This reduction would necessitate the use of a sanative at all treatments. This supposition is based on Spinks' (1950) work with Antrycide chloride with which drug the availability is reduced by its relative insolubility. With this compound the primary blood levels are low, while the latter levels are relatively unaffected.

A regime trial is at present being planned and it is hoped to include some of these ideas on drug regimes in this trial, which will also include costing.

SUMMARY

The possible reasons for the development of drug resistance in trypanosomes during chemoprophylaxis are discussed and some possible solutions to prevent this are proposed.

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A REVIEW OF BOVINE TRYPANOCIDAL DRUG TRIALS OF THE UGANDA VETERINARY DEPARTMENT

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Three trials have been carried out in Uganda in the last three years. One of these, reported in part at the ISCTR Meeting at Conakry (Cawdery, 1962), dealt with local tolerance. The other two were trials under field challenge. These trials will be reported in detail elsewhere, but the main information will be given here as a subject for discussion by the Committee.

Both trials of drugs under field challenge were conducted in the same area in Ankole in south-west Uganda. The geographic position was 0°36′ South and 30°48′ East. *Glossina morsitans* West. was the only species of tsetse present.

TRYPANOSOME RISK

During the first trial, fly rounds were done and the infection rate of the tsetse was established. Unfortunately this was not possible during the second trial, but control groups of cattle were used in both trials for comparative purposes (Table I). Berenil at 10·5 mg./kg. was used to treat control cattle when they showed a patent infection (Tennant and Cawdery—in preparation).

The details of these investigations are summarised below. The method of estimating challenge from tsetse fly rounds and infection rates is that of Whiteside (1957).

A fly round apparent density of 40 was found. The infection rate obtained by dissection (Lloyd and Johnson, 1923) was 20·5%. The challenge index was therefore 820.

Table I.—Comparison of the control groups in each trial
All cattle showed patent infections in both trials

	No. of control cattle	Mean number of days to patent infection after each Berenil treatment	Total No. of cattle in trial
First trial	8	30·7	65 (max)
Second trial (not complete) .	9	aprox. 42	115

CATTLE

All cattle used in these trials were Ankole steers approximately 2–4 years old. No cattle with Antrycide Prosalt lumps were used but it is likely that many of these cattle had received Antrycide methylsulphate or Berenil at some previous time. These drugs were, until very recently, the only drugs in use for trypanosomiasis in Uganda.

In the first trial, cattle in the prophylactic groups were withdrawn when they showed a patent infection. In the second trial they were treated with Berenil at 10·5 mg./kg. after showing a patent trypanosome infection and allowed to remain in the trial area. Thereafter they received monthly Berenil injections at 10·5 mg./kg. This retention in the trial area was to maintain the herd size and to try and avoid increasing the challenge by reducing the number of animals available to the tsetse. The monthly Berenil was used to stop the development of drug resistance.

RESULTS

As the second trial is not yet finally complete results are given up to twenty-five weeks for comparative purposes. These are tabulated below.

Table II.—The results of two comparative prophylactic drug trials in Ankole
First Trial (from Tennant and Cawdery—in preparation)

Drug, route of administration and vehicle	Dosage rate	No. positive (25 weeks) No. in group	Shortest protective period
M & B 4180 2% Aqueous solution intramuscularly	2 mg/kg	15/20	17·5 weeks
M & B 4404 14·7% Suspension in lanolin + olive oil subcutaneously	4 mg/kg	3/6	20 weeks
M & B 4596 10% Aqueous suspension intramuscularly	5 mg/kg	3/6 at 15 weeks. Remainder removed from trial	13 weeks
M & B 4596 10% Aqueous suspension subcutaneously	5 mg/kg	3/8 at 16 weeks. Remainder removed from trial	14·5 weeks
M & B 4596 10% Aqueous suspension intramuscularly	10 mg/kg	2/4 at 16 weeks. Remainder removed from trial	14·5 weeks
M & B 4427 10% Aqueous suspension intravenously	10 mg/kg	4/6 at 19 weeks. Remainder removed from trial	16 weeks
Antrycide Prosalt O.F. 23% Aqueous suspension subcutaneously	11·7 mg/kg	6/6 at 8 weeks	4 weeks
Second Trial (not complete) **1. Prothidium bromide**			
2·5% w/v Aqueous solution subcutaneously	2 mg/kg	6/10	16 weeks
10% w/w H.V.B.* subcutaneously	2 mg/kg	4/10	16 weeks
10% w/w H.V.B.* subcutaneously	1 mg/kg	4/10	16 weeks
2. Antrycide Prosalt			
23% w/v Aqueous solution subcutaneously	11·7 mg/kg	10/10	6 weeks
23% w/w H.V.B.* subcutaneously	11·7 mg/kg	6/8	6 weeks

Drug, route of administration and vehicle	Dosage rate	No. positive (25 weeks) No. in group	Shortest protective period
3. Samorin (isometamidium)			
5% w/v Aqueous solution intramuscularly	1 mg/kg	4/10	16 weeks
5% w/v Aqueous solution intramuscularly	0·5 mg/kg	4/10	17 weeks
10% w/w H.V.B.* subcutaneously	1 mg/kg	5/10	17 weeks
10% w/w H.V.B.* subcutaneously	0·5 mg/kg	6/10	16 weeks
4. Samorin/Prothidium mixtures			
2·5% w/v Aqueous solution intramuscularly	0·5 mg/kg each drug	6/10	17 weeks
10% w/w H.V.B.* subcutaneously	0·5 mg/kg each drug	2/8	20

* H.V.B. = High viscosity base.

DISCUSSION

There is one thing which stands out in these trials and that is the apparent lack of difference in the protective period between different dosage rates of the same drug, different routes of administration and different vehicles with the possible exception of the Prothidium/Samorin mixture in a high-viscosity base. This lack of effect of doubling the dosage rate was also noted by Smith (1959) though not by Lyttle (1960) in trials using grade cattle. Lyttle, however, showed no difference between 2 mg./kg. and 4 mg./kg. of Prothidium in zebu cattle. Robson and Cawdery (1958) showed a difference at these dosage rates of Prothidium in zebu cattle, but only a slight difference between 5 mg./kg. and 10 mg./kg. of Homidium suraminate in zebu cattle. This effect noticed in these trials and by other authors may be of considerable importance in the field utilisation of these drugs. For example, it may be possible to dose cattle in a limited number of weight ranges. A possible example of this is shown for Prothidium bromide in Table III.

Table III.—Suggested doses and weight ranges for Prothidium and the resultant dosage rate ranges

Dose	Weight range	Dosage rate range
100 mg	40– 75 kg	2·5 –1·33 mg/kg
200 mg	75–150 kg	2·67–1·33 mg/kg
400 mg	150–300 kg	2·67–1·33 mg/kg
800 mg	300–600 kg	2·67–1·33 mg/kg

In a tablet form this would mean that both the stock owner and veterinary assistant would have little trouble in ascertaining the appropriate dose and it would reduce the possibility of malpractice, and thereby reduce the ever-present danger of drug resistance developing in the trypanosomes.

It was hoped that the high-viscosity base for injection of the irritant phenanthridines would, in addition to improving local tolerance, also enhance the protective period of the drug by creating a local depot from which the drug would be slowly leached and thereby maintain a higher blood and tissue level than was possible with the normal aqueous solution or suspension. Though earlier work indicated that this might happen, the most recent trial does not substantiate it. Possible reasons are discussed in a second paper to this Conference.

The main reasons, therefore, for using a high viscosity vehicle for the administration of trypanocides are: firstly, an improvement in local tolerance to the extent that a drug such as Samorin may be given subcutaneously and, secondly, it is a stable preparation which does not require field preparation. Unfortunately, no costings have been worked out but it is suspected that the additional cost of such preparations may be too high to warrant their use unless there was a very big demand.

It is interesting to note that the limited amount of work noted in this report on the routes of administration shows no indication of a preferential route in relation to the protective period, though improved local tolerance has been noted by some workers (Finelle and Yvore, 1962). A possible reason for this is discussed in a second paper to this Conference.

SUMMARY

(1) The results of field trials in Uganda are given.

(2) These results are discussed in relation to their possible effect on field utilisation.

(3) The effect of high-viscosity bases on the protective period and their advantages are discussed.

(4) The effect of route of administration is noted.

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DIAGNOSIS OF TRYPANOSOMIASIS IN CATTLE

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This communication presents evidence for the evaluation of tests currently available for the diagnosis of trypanosomiasis in cattle. The diagnostic tests discussed are as follows: microscopical examination of stained blood films, sub-inoculation of blood into susceptible animals and serological tests. The serological tests are an agglutination test (Cunningham and Vickerman, 1962) and an agar-gel double-diffusion test (Cunningham, 1961).

1. The examination of stained blood films

In July 1959, four blood films were prepared from each of 211 cattle in poor condition (representing 5% of cattle in the district) in the Lower River district of Nyasaland. One batch of 211 slides was sent to EATRO for examination, and each of the three other batches were examined in other laboratories. At EATRO, the blood films were stained with Giemsa and examined under oil immersion until positive, or until 200 fields had been searched. Fifteen slides were found positive. In the three other laboratories, a total of twenty-three slides were found positive. Of these twenty-three slides, only ten were found positive at EATRO, and the remaining five slides positive at EATRO were negative at the other three laboratories. The thirteen slides found negative at EATRO and positive at the three other laboratories, were re-examined until positive or until 1,000 fields had been searched. Single trypanosomes were found on seven of these slides, after an average of 700 fields had been thoroughly searched. These results point out obvious difficulties in arriving at a proper estimate of the incidence of infection in the sample examined by this method.

A zebu cow, experimentally infected with *T. vivax*, died 116 days after inoculation of infective material. Blood films were irregularly examined initially, and none was found positive. By the seventy-sixth day of infection, however, the animal was losing condition and daily blood films were examined from then until the cow died. Of the forty blood films examined, only four were positive. While it is unwise to draw conclusions from results obtained from one animal, it is thought probable that similar instances will occur naturally, and it is probable that the examination of blood films is an inefficient diagnostic procedure for *T. vivax* infections in cattle.

2. Comparison of blood film examination with inoculation of blood into mice

Thick blood films from four zebu cattle experimentally infected with *T. brucei* Subgroup organisms were examined daily for eighty-three days, starting on the day after infection. Half a ml. of blood from each of these animals was inoculated into each of four mice at weekly intervals for twelve weeks. Of the 332 slide examinations, twenty-four were positive, and forty-seven of the forty-eight inoculations into mice were positive.

Thick blood films were obtained from 203 cattle exposed to natural infection

at Alego, Nyanza, Kenya, and at the same time 0·5 ml. of blood from each animal was inoculated intraperitoneally into each of two mice. Forty-three of the cattle were found to be infected with *T. brucei* Subgroup organisms on inoculation into mice and, of these, only seven were positive on examination of thick blood films. Six cattle were positive on examination of thick films and failed to infect mice on intraperitoneal inoculation of 0·5 ml. of blood. It was not possible to identify the species of trypanosomes from the thirteen cattle found positive by examination of thick blood films.

In a small survey carried out in Ankole, on twenty-nine clinically suspect cattle, a total of twelve were found to be positive by examination of thick blood films and by inoculation of 0·5 ml. blood from each animal into each of two mice. Of the twelve positives, nine were positive on slides and six were positive in mice. Of the mouse positives, three were negative on slides and of the slide positives six were negative in mice. Three were positive both in mice and on slides. Of the twelve positives, eleven were *T. congolense* and one was *T. vivax* (negative in mice).

Godfrey and Killick-Kendrick (1961) have recently carried out a survey of trypanosomiasis in cattle in the Donga Valley, Benue Province, Nigeria. The diagnostic procedures used were examination of thin and thick blood films and the inoculation of blood from the cattle into rats. In the diagnosis of the disease by examination of blood films, they found thick films more efficient than thin films, but recommended that thin films be examined for identification of the organisms. In the diagnosis of *T. vivax* infections, the inoculation of rats was of little value. In the diagnosis of *T. congolense* infections, many positive blood films were negative in rats and vice versa. In the diagnosis of *T. brucei* infections, while no blood films were positive, many rats became infected subsequent to inoculation of blood.

3. Diagnostic trial with serological tests

The herd of cattle used in this investigation is on the landward edge of the fly belt on the north-east coast of Lake Victoria. *T. brucei* Subgroup, *T. vivax* and *T. congolense* are enzootic in the area. For several years the herd had been under Antrycide Prosalt prophylaxis, but for at least four months at the end of 1961 no treatment was administered. The following survey was carried out approximately 2½ months after Antrycide prophylaxis was resumed, i.e. after two treatments with the drug. The herd consists of 114 Shorthorned zebu cattle of all ages. Blood from each animal was obtained from the jugular vein, using a sterile 5-ml. syringe. Three ml. of this blood was inoculated into a sterile bijou bottle, a thick blood film prepared, and the remainder inoculated I.P. into two mice. In every case the time between collection of blood and inoculation into mice was less than one minute. (The same syringe, with a small-bore needle substituted, was used to inoculate the mice.) Serum was separated twenty-four hours after collection of the blood and stored at approximately —20° C. All sera were tested against three antigenic variants of the *T. brucei* Subgroup for agglutination. All sera were tested against one antigenic type of the *T. brucei* Subgroup for precipitation in the agar-gel double-diffusion test. The thick blood films were stained with Giemsa, and examined under oil immersion until positive or until 200 fields had been examined. Tail blood from the mice was examined at daily intervals for fifteen days and at weekly intervals thereafter until the sixty-fourth day after inoculation.

The table shows the numbers of positive animals detected by the different methods.

The total number of positives in mice was fourteen; by thick film examination, six; by agglutination test, twenty-eight; by agar-gel double-diffusion precipitation test, seventeen. Of the fourteen infections found by inoculation of blood into mice, three were of the *T. brucei* Subgroup and eleven of the *T. congolense* Group. *T. theileri* was found on one thick film.

The number of positive animals detected by the different methods

Agglutination alone	18
Agar-gel diffusion alone	11
Thick film alone	2
Mice alone	2
Thick film and mice	2
Thick film and agar-gel diffusion	1
Agglutination and mice	7
Diffusion and mice	1
Agglutination and agar-gel diffusion	2
Thick film, agar-gel diffusion and mice	1
Agglutination, agar-gel diffusion and mice	1
Total positive animals	48

In a similar survey of fifty cattle from the EATRO farm, none was found positive by inoculation of blood into mice, by thick film examination or by the agglutination test, but five gave positive results in the agar-gel double-diffusion precipitation test. Of these five, four were purchased from outside and one was born on the farm.

CONCLUSIONS

Diagnosis of *T. vivax.* infections

The microscopical examination of blood films is the only test available, and is considered to be very inaccurate.

Diagnosis of *T. congolense* infections

Microscopical examination of blood films and subinoculation of blood into mice are complementary, and should be used together where possible.

Diagnosis of *T. brucei* Subgroup infections

Microscopical examination of blood films is of very limited value, while subinoculation of blood into mice and serological tests probably give fairly accurate results. A positive serological test, however, need not necessarily imply infection, since antibodies probably remain detectable long after curative treatment has been carried out.

It is considered that the diagnostic tests in use at present are probably adequate in establishing the presence or absence of the disease on a herd basis, but are of limited value for accurate diagnosis of individual infected animals. Until accurate and reproducable diagnostic tests are developed, it will not be possible to carry out comprehensive surveys on the incidence of trypanosomiasis in domestic and wild animals.

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THE BIOLOGICAL CONTROL OF THE ANTIGENIC CHARACTERS OF A STRAIN OF TRYPANOSOMES

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INTRODUCTION

Protozoologists studying African trypanosomiasis are often warned against making inferences from experiments with old syringe-passaged laboratory strains on the nature of phenomena occurring in tsetse fly-transmitted strains. Cyclical transmission by tsetse flies may have a profound effect on certain characters of trypanosomes. Bruce (1914) was one of the earliest workers to recognise this danger and he suggested that much of the confusion then prevalent in the classification of African trypanosomes was due to the use of strains which had been maintained for some years by frequent sub-passage in laboratory animals. The morphology and pathogenicity of such strains changed considerably and he advocated that only newly isolated strains or strains transmitted by tsetse flies should be used in such work.

The importance of working with tsetse-transmitted strains has frequently been re-emphasised, particularly in experimental chemotherapy, where laboratory findings are liable to be applied in the field. It has been shown, for example, that strains of *T. rhodesiense* retain a natural resistance to certain arsenical drugs when they are transmitted by tsetse flies, but lose it rapidly when transmitted repeatedly by syringe (Yorke and Murgatroyd, 1935; Murgatroyd and Yorke, 1937) and Unsworth (1954 a, b) has described differences in the sensitivity of syringe-passaged and cyclically transmitted strains of *T. vivax* to homidium bromide (B. vet. C.). Apart from preserving such natural strain differences of drug sensitivity, cyclical transmission can also affect the persistence of acquired drug resistance. For example, tryparsamide resistance in *T. brucei* and *T. gambiense* is retained, but suramin resistance in *T. gambiense* is reduced during transmission of strains by tsetse flies (see Bishop, 1959) and resistance to certain cattle trypanocides is lost in strains undergoing cyclical transmission (MacOwan, 1958).

Although such results suggest that cyclical transmission may have some effect on the antigenic characters of strains of trypanosomes, and this possibility has often been discussed, few studies of immunological phenomena in tsetse-transmitted strains have been made. Desowitz (1960) drew attention to the disproportionately small amount of work which has been done in general on immunity of game and domestic animals to trypanosomiasis, as opposed to the wealth of publications on anti-trypanosomal immunity in laboratory rodents. There is an even greater scarcity of publications on the effects of cyclical transmission on the antigenic characters of strains.

Broom and Brown (1940) and Gray (1962 a) have studied aspects of this latter problem with strains of *T. brucei* and have shown that serological differences among variants of a strain, which develop as a result of antigenic variation, are reduced during cyclical transmission, so that cyclical substrains of one strain

transmitted by different tsetse flies resemble each other closely. By extending this work on antigenic variation in cyclically transmitted strains, it has been possible to gain some knowledge of how the antigenic characters of a strain may be maintained in nature when it is transferred from one vertebrate host to another by syringe or tsetse fly and these findings form the basis of the present paper.

It should be emphasised that many of the observations on which the following discussion is based have been limited to the variable agglutinogenic trypanosomal antigens. Other antigens may not behave in the same way. Furthermore, these observations have been made, in many instances, only on strains transferred from one host to another after maximum periods of infection of thirty days. It is possible that greater, or even permanent, antigenic changes may occur in strains during more chronic infections and in such circumstances their antigenic characteristics may not be controlled by the means described.

The capacity of trypanosomes for antigenic variation

The potential of trypanosomes for antigenic variation has been a major obstacle to serological classification of strains and to immunisation against trypanosomiasis for many years. The main features of antigenic variation are summarised briefly in the following paragraphs, to give some idea of what is known before the problem is discussed in the light of the results of the present experiments.

Franke (1905) first showed that trypanosomes may change immunologically in an infected animal and Ritz (1916) soon demonstrated in experiments with a clone of *T. brucei* that a single trypanosome can give rise to a strain of organisms producing many different antigens. Further work has established that series of trypanosomal antigens develop during infections, under the influence of antibody produced by infected vertebrate hosts. New antigens develop at intervals of approximately two to three days from the beginning of an infection until the animal dies. Individual organisms from a strain produce a similar range of antigens, but the range of antigens produced by one strain differs from the ranges of antigens produced by other strains. The change of antigenic type of a strain consists of a gradual alteration of the proportions of the developing and existing antigens. It is possible for two or more antigens to develop at the same time, so that variants or substrains may owe their serological characteristics to a combination of those of several antigens.

Considerable uncertainty exists about the number of variable antigens which may be produced by a given strain. Ritz (1916) found twenty-two different antigenic types among variants of a strain of *T. brucei* and did not believe that the variability of his strain was exhausted; similarly, Lourie and O'Connor (1937) isolated thirteen different variants of a strain of *T. rhodesiense*, while Osaki (1959) has described twenty-three different types of a strain of *T. gambiense*. These findings, however, were probably dependent on the prevailing experimental conditions. In the present experiments, new antigens developed at frequent intervals from the time an animal was first infected until it died, and the number of antigens produced seemed to be limited only by the period the infected animal survived.

As a result of the variability of trypanosomal antigens we still have no idea of the number of antigenically different strains of any of the species of African trypanosomes which exist in the field.

The tendency for a strain to produce one characteristic antigen

Although it is easy to gain an impression from the literature of unlimited antigenic diversity in trypanosomes, there seems to be a tendency for each strain to produce one particular antigen.

Substrains of a strain transmitted by different tsetse flies are serologically closely related to each other (Broom and Brown, 1940; Gray, 1962 a). Such cyclical substrains have an antigen in common which has been called for convenience the basic strain antigen (Gray, 1963). Studies of the antigenic similarity of such substrains have shown that tsetse flies which ingest trypanosomes with variant antigens of a strain, transmit substrains with either the basic strain antigen only, or with a mixture of the basic and variant antigens.

When serological variants of a strain are transferred from infected animals to new, non-immune hosts by syringe, there is a similar tendency to revert to one antigenic type; trypanosomes with the basic antigen, associated with cyclical substrains, appear in the blood of each newly infected animal as soon as antibody to the infecting variant has been produced.

In both the tsetse fly and the hitherto unexposed host there is presumably an absence of specific antibody to the basic antigen of the strain, thus providing an environment which favours its development.

Earlier authors have also noted a tendency for serological variants of strains to revert to a " parent " type of organism. Lourie and O'Connor (1937) and Soltys (1963) have reviewed reports of relapse strains reverting to " parent " antigenic types during repeated syringe passage at short intervals in laboratory rodents, a procedure again calculated to minimise the effect of the immune response of the host on the strain, and Osaki (1959) has described a preponderance of one particular relapse strain among many variants isolated in studies of antigenic variation in *T. gambiense.*

It seems, therefore, that the innate tendency for trypanosomes of a strain to produce one particular antigen in a suitable environment is fairly well established.

The stability of the basic antigen of a strain

Variation from the basic strain antigen does not seem to occur in the absence of specific antibody. When a tsetse fly ingests trypanosomes with the basic antigen, the antigen persists in the organisms during the period required for cyclical development and for the remainder of the life of the fly. Substrains isolated from animals infected by such flies are serologically indistinguishable.

The basic antigen persists in the strain in an infected animal for seven to eight days after the animal is bitten by an infective fly, that is, until the animal produces specific antibody.

Results obtained up to the present time indicate that basic antigens are characteristic of strains. Broom and Brown (1940) found that cyclical substrains of three strains of *T. brucei* were antigenically distinct and similar observations have been made with a further three strains of this species (Gray, 1963). Early hopes that the distinctive antigenic nature of cyclical substrains might provide a simple means of classifying strains may have been disappointed, however, by the discovery that many such substrains possess variant antigens as well as the basic strain antigen, but further work is in progress on this aspect of the problem. It is still possible that the basic antigen may be of value in tracing strains in future epidemiological studies of trypanosomiasis.

Organisation of antigenic variation

The classical investigations of antigenic variation in trypanosomes with syringe-passaged strains have revealed little evidence of organisation in the sequence in which the antigens develop during an infection. Such experiments have usually been limited to determinations of the number of different variants which arise from a single stock of a strain when it has been used to infect several different hosts. Some authors have shown that a wide range of different variants develops from one stock of a strain (see Lourie and O'Connor, 1937), but others have found that most of the variants which develop in such experiments fall into a very limited number of discrete antigenic types (Leupold, 1928; Osaki, 1959; Inoki, 1960). However, results obtained in the present experiments indicate that the development of the antigens of a strain may not be such a random process as the earlier work suggested.

During a study of antigenic variation in an old laboratory strain of *T. brucei*, passaged at frequent intervals by syringe in rabbits, a distinct pattern of antigenic variation was detected which was so constant that it was possible to predict which antigens would develop from a given stock of the strain (Gray, 1962 b). When this study was extended to clone strains of *T. brucei*, it was found that certain antigens always developed at an early stage of infection when serological variants of the clones were introduced into new hosts by syringe. Such antigens have been described as predominant antigens of the strain.

An even greater degree of organisation in the development of antigens of a strain was observed in animals infected with a strain of *T. brucei* transmitted by tsetse flies. The antigens of the strain developed in an identical sequence in the early stages of infections of animals bitten by different tsetse flies; at later stages of infection, however, there was considerable variation in the sequence in which antigens developed in different animals, but many of the antigens developing in each infection were identical. Most of the antigens, including the basic antigen, which developed in these tsetse fly-induced infections also developed at an early stage of infection in animals infected with serological variants by syringe and, by analogy with findings with clone strains, may also be described as predominant antigens of the strain.

SUMMARY

It seems that the mode of transmission of a strain of trypanosomes, that is, whether it is cyclically transmitted by tsetse flies or mechanically transmitted by biting flies, a phenomenon which is simulated in many experiments by syringe passage, is unlikely to be an important factor in controlling its antigenic character. The basic strain antigen associated with cyclical substrains develops at an early stage of each new infection induced by either means, while other predominant strain antigens develop in alternate hosts when a strain is passaged through a series of animals by syringe, thus ensuring the continuity of strain antigenic characteristics.

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SEROLOGICAL INVESTIGATIONS IN CATTLE INFECTED WITH *TRYPANOSOMA BRUCEI* SUBGROUP ORGANISMS

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INTRODUCTION

The investigations described in this report were carried out to attempt to answer the following questions concerning *T. brucei* group infection in cattle:

(1) Will cattle infected with a large number of trypanosomes and treated with Berenil three days after infection produce agglutinins against the inoculated organisms ?
(2) Will cattle treated as in (1) above resist challenge with homologous organisms three weeks later ?
(3) What will be the antibody response of cattle treated as in (2) above, as judged by the agglutination test ?
(4) Will cattle treated as in (1) above, but inoculated with a different antigenic variant of the same strain, produce homologous antibodies only ?
(5) What will be the effect of simultaneous inoculation of several antigenic variants of the same strain into one animal, with regard to antibody response ?
(6) What will be the antibody response of an animal treated as in (5) above, and challenged three weeks later with homologous organisms ?
(7) If cattle treated as in (4) above do produce homologous antibodies only, can tsetse flies which feed on them be identified using the agglutination test ?

MATERIAL AND METHODS

Trypanosomes

T. brucei Plummer and Bradford Subgroup (Hoare, 1956), designated EATRO-3, preserved at approximately —80° C. as described by Cunningham *et al.* (1963).

EATRO-3 was inoculated into Bullock No. 480. Subinoculations of blood from Bullock 480 at intervals of one, five and nine weeks after inoculation of EATRO-3 were made into mice.

Investigations revealed that 480/1, 480/5 and 480/9 were antigenically heterologous, agglutinins prepared in rats gave agglutination with the homologous antigen only. 480/1, 480/5 and 480/9 were used in the present investigation. Capillary lymph tubes, and ampoules containing 1 ml. trypanosome suspension were preserved at —80° C. as described by Cunningham *et al.* (1963).

Cattle

Four East African shorthorned zebu from the EATRO herd were used. Before the investigation they were found to be free from trypanosomiasis as judged by inoculation of blood into mice, examination of stained and wet blood films and absence of agglutinins in their sera.

AGGLUTINATION TEST

The test described by Cunningham and Vickerman (1962) was used.

Methods

One ampoule each of 480/1, 480/5 and 480/9 was thawed rapidly from —80° C., and each added to 6 ml. of B.S.-2.

The number of infectious organisms was established for each of the three suspensions, using Lumsden's infectivity titration technique (Lumsden *et al.*, 1963).

The four cattle were inoculated intravenously as follows:

Animal No.	Trypanosomes	Volume	Log. No. ID_{61}'s
23	480/1	4·5 ml.	
167	480/5	4·5 ml.	
985	480/9	4·5 ml.	
59	480/1	1·5 ml.	
	480/5	1·5 ml.	
	480/9	1·5 ml.	

On the third day after infection, animals 23, 167 and 985 were treated with Berenil, 7 mg./kg. intramuscularly. Before treatment, these animals were parasitaemic. On the twenty-fourth day after infection the cattle were challenged, as shown above.

Serum was obtained from each animal each day for six days, then at two- or three-day intervals until the twenty-fourth day. After challenge on the twenty-fourth day serum was obtained each day for six days. Serum was stored at approximately —25° C. until used in the agglutination test.

Groups of *G. morsitans* were fed on each of the four cattle, seventeen days after infection. A group of flies were also fed on a non-infected cow. Twenty-four hours after feeding the abdominal contents of the flies were smeared individually on filter paper. The smears were randomised in a series 1–50 and extracted in 0·5 ml. B.S.-2 for approximately thirty minutes at 25° C. Each extract was tested against antigens 480/1, 480/5 and 480/9 for agglutination.

Results

Each of the questions posed in the introduction will be dealt with separately.

(1) Yes. Each of the three treated cattle produced high titre agglutinins.

(2) Yes. As judged by subinoculation of blood into mice, all three cattle resisted challenge with homologous organisms.

(3) A slight increase in agglutinins was observed.

(4) Slight cross agglutination was obtained with 480/1 and 480/5 using serum from animal 167, but high titres were recorded against homologous antigens only.

(5) The animal inoculated with the three antigenic variants produced high titre agglutinins against all three variants.

(6) A slight increase in agglutinins was observed against each of the antigenic variants.

(7) The extracts from the fed tsetse flies were all, without exception, correctly ascribed to one of the four " labelled " animals, or to the negative control animal.

Acknowledgements

Miss Fromentin acknowledges the receipt of a grant from the WHO.

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Acknowledgements

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UTILISATION DES GLUCIDES ET DE LEURS PRODUITS DE METABOLISME PAR *TRYPANOSOMA EVANSI* ET *TRYPANOSOMA BRUCEI*

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Assez peu de chercheurs ont étudié spécialement le métabolisme glucidique de *T. evansi* et *T. brucei.*

Krijgsman [5] dans un important travail donne des précisions très intéressantes; à son avis, *T. evansi* est capable d'utiliser 3 sucres: glucose, fructose et mannose. Par contre, il ne possède aucune des diastases suivantes: amylase, maltase, saccharase et lactase.

Krijgsman pense, sans le prouver, que vraisemblablement le résultat du catabolisme glucidique est comparable à celui de *T. equiperdum*, c'est-à-dire, constitué avant tout par l'acide pyruvique (Reiner et Smythe [7], Chen et Geiling [3].)

Cette dernière opinion est en opposition avec celle de Kligler, Geiger et Comaroff [4] qui pensent que l'impossibilité de réaliser des cultures de *T. evansi* est due à la formation massive d'acide lactique.

Par contre, Marshall [6] dans une très intéressante publication, étudiant le métabolisme du glucose dans ses rapports avec l'action des trypanocides, dose les produits terminaux et constate qu'il se forme surtout de l'acide pyruvique et très peu d'acide lactique.

Enfin, pour von Brand [9] *T. brucei* est capable d'utiliser: glucose, mannose, maltose, fructose et galactose, respectivement dans les rapports 100–86–50–21–9.

En tenant compte de ces travaux, nous avons expérimenté différents glucides et polyosides ainsi que quelques produits de leur métabolisme.

Nous avons d'abord recherché spécialement chez *T. evansi* quelle était d'une part, l'action de ces glucides sur la survie, et d'autre part, l'importance de la libération d'acide pyruvique résultant de leur éventuel catabolisme.

Puis, par une technique totalement différente de la précédente, nous avons fait une étude comparative de 2 souches de *T. evansi* (dont l'une résistante à la Lomidine) et d'une souche de *T. brucei.*

MATERIEL ET METHODES

La souche de *T. evansi* a été prélevée en décembre 1961 sur un âne de Fort-Lamy. Elle a été conservée depuis par passages sur rats et cobayes. Une race chimio-résistante a été créée par traitements répétés et progressifs à la Lomidine, des rats parasités.

La souche de *T. brucei* provient d'un chien malade qui nous fut présenté en janvier 1964. Cette souche est entretenue depuis sur rats et cobayes.

L'expérimentation a été conduite selon 2 techniques:

1° Sur milieux diphasiques mis en tubes à essais

La phase solide est de la gélose physiologique à 2 pour cent qui sert de support

à la substance étudiée. Nous utilisons toujours au moins 5 tubes pour chacune d'elles car un lavage défectueux peut sensiblement modifier les résultats et on a donc intérêt à faire une moyenne.

La phase liquide est différente selon que l'on veut expérimenter des glucides ou leurs produits de métabolisme.

a) Pour les glucides on utilise un milieu au sang de cheval pratiquement dépourvu de glucose. Sa composition est la suivante:

— sang de cheval 10 ml
— liquide " Roche " en solution à 1 pour cent 1 ml
— eau distillée 90 ml

hémolyse pendant 1 heure à la température du laboratoire, puis addition de:

— phosphate bipotassique 1 g
— chlorure de sodium 0 g 50

ajuster si nécessaire à pH 7,4 avec du phosphate monopotassique.
Filtration sur papier puis sur Seitz.

b) Les produits provenant du métabolisme des glucides étant toxiques on utilise le milieu suivant:

— sang de cheval 10 ml
— liquide " Roche " en solution à 1 pour cent 1 ml
— eau distillée 90 ml

hémolyse

— phosphate bipotassique 1 g
— glucose 2 g

ajuster si nécessaire à pH 7,4 avec du phosphate monopotassique.

On ensemence des milieux avec du sang de rat parasité de façon qu'une fois l'opération terminée, la quantité de trypanosomes soit comprise entre 20.000 et 40.000 au mm^3.

Après une légère agitation on répartit dans les tubes d'expérience à raison de 2 ml environ pour chacun d'eux.

Les tubes contenant le milieu sans glucose sont maintenus à 25° pendant 6 heures. Au bout de ce temps, on récolte la phase liquide et on pratique une numération des trypanosomes à l'hématimètre ainsi qu'un dosage de l'acide pyruvique.

Nous utilisons pour ce dernier la méthode de Caron et Raquet modifiée par nous-mêmes.

Les tubes contenant le milieu glucosé sont maintenus pendant 20 heures à 25°. La suite des opérations est la même que précédemment sauf le dosage de l'acide pyruvique qui ne présente ici aucun intérêt.

Afin de limiter les causes d'erreur, l'ensemble de l'expérimentation est répété plusieurs fois. Il n'est pas nécessaire de travailler d'une façon absolument aseptique, car la durée d'observation est trop courte pour permettre à de légères souillures de se développer. C'est ainsi que les glucides non stériles sont introduits dans la gélose à une température de 50 à 60° ce qui évite une hydrolyse partielle des polysaccharides. Cependant, il est conseillé d'utiliser du matériel passé au four Pasteur.

Cette méthode n'est pas facilement appliquable à *T. brucei* car les formes proventriculaires qui prennent naissance sont beaucoup plus résistantes et faussent les résultats.

2° Test de mobilité

Il nous a été inspiré par un travail de Schern [8] datant de 1925. Cet auteur avait remarqué que lorsqu'on met en présence de sérum, des trypanosomes immobilisés, ceux-ci reprennent leur mouvement au bout d'un quart d'heure environ. Le sérum contient donc une substance énergétique que Schern trouva être le glucose.

En généralisant, nous avons pensé que tout corps énergétique pouvait provoquer une mobilisation du parasite si ce dernier était capable de l'utiliser.

Cela s'est confirmé et nous avons élaboré la technique suivante:

Du sang de rat très fortement parasité est dilué au 1/20 environ dans le milieu synthétique suivant:

— phosphate bipotassique	1 g
— chlorure de sodium	0 g 25
— eau distillée	100 ml
— phosphate monopotassique	— Q.S. pour obtenir pH 7,4.

Cette suspension est répartie à raison de 2 ml dans autant de tubes à essais qu'il y a de substances à étudier. On ajoute alors dans chaque tube la substance correspondante à raison de 2 gouttes d'une solution à 1 pour cent.

Un tube ne contenant que la suspension sert de témoin et on y constate une immobilisation rapide des trypanosomes, ces derniers ayant épuisé le peu de glucose qui pouvait se trouver dans le sang.

Après immobilisation complète dans la suspension témoin on dépose sur une lame 2 gouttes (témoin et corps à étudier) qu'on recouvre d'une lamelle. Il est alors aisé de se rendre compte à l'examen microscopique si la substance a une action énergétique, c'est-à-dire si elle permet une réanimation des trypanosomes.

Avantages de la méthode: Ils sont multiples.

a) Le travail ne demande aucune précaution de stérilité.
b) Elle permet de pratiquer très rapidement de nombreux examens sur le même prélèvement de sang.
c) Elle met en jeu une quantité très faible de réactif de l'ordre du milligramme.

Inconvénients:

a) Il est nécessaire d'avoir un sang très riche en trypanosomes (au moins 600.000 par mm^3) de façon que le glucose présent soit rapidement utilisé. Cette difficulté peut cependant être éliminée en utilisant une suspension de trypanosomes lavés.
b) Certaines substances classées énergétiques ne peuvent l'être par l'intermédiaire d'une diastase sanguine, par exemple le maltose. Cette méthode ne permet pas de déterminer l'origine de la diastase.

RESULTATS ET DISCUSSION

Les résultats sont résumés dans les tableaux I et II (milieux diphasiques) ainsi que III (test de mobilité). Dans ce dernier cas, outre les glucides et leurs produits de métabolisme, nous avons expérimenté des acides aminés glucoformateurs.

T. evansi utilise avec dégagement notable d'acide pyruvique: glucose, mannose, fructose, maltose et glycogène (tableau I).

Aucun pentose n'est actif, cependant, avec tous les glucides et polyosides étudiés et non actifs, le nombre de trypanosomes survivants est toujours légèrement supérieur à celui observé chez le témoin.

Le maltose et le glycogène doivent subir une hydrolyse pour être utilisés. Nous avons mis en évidence une maltase et une glycogénase dans le sang de rat. Nous pensons que *T. evansi* est dépourvu de ces diastases puisqu'il ne possède aucune réserve glucidique. En définitive, il semble que seuls glycérine, glucosamine,

Tableau I

No.	Substance étudiée	Nbre de trypano. par mm³ après 6 h	Pyruvate de Na au mg. par ml	Pyruvate de Na réellement dû au catabolisme de la substance étudiée
1	Témoin . . .	4.200	0,02	
2	Glycérol . . .	5.500	0,06	0,04
3	Arabinose . .	5.000	0,02	0
4	Ribose . . .	5.500	0,03	0,01
5	Xylose . . .	6.000	0,03	0,01
6	Lyxose . . .	6.200	0,03	0,01
7	Glucose . . .	23.400	0,4	0,38
8	Lévulose . .	21.800	0,2	0,18
9	Mannose . .	23.400	0,2	0,18
10	Galactose . .	8.000	0,02	0
11	Rhammose . .	6.800	0,04	0,02
12	Maltose . . .	23.000	0,3	0,28
13	Lactose . . .	5.600	0,04	0,02
14	Tréhalose . .	5.200	0,04	0,02
15	Saccharose . .	6.000	0,02	0
16	Raffinose . .	4.800	0,05	0,03
17	Inuline . . .	5.000	0,03	0,01
18	Glycogène . .	20.600	0,15	0,13

Le nombre de trypanosomes au départ de l'expérience était de 38.000 au mm³.

Tableau II

Série No.	Substance étudiée	Nbre de trypanosomes au mm³ après 20 h
1	Témoin	1.080
2	Acide pyruvique 1/10 cc	0
3	Acide oxaloacétique 100 mg	280
4	Acide citrique 100 mg	920
5	Acide α cétoglutarique	200
6	Acide succinique 100 mg	400
7	Acide fumarique 100 mg	420
8	Acide malique	320

Nombre de trypanosomes au début de l'expérience : 40.000 au mm³.

fructose et mannose sont utilisés directement. Ces 4 derniers corps ont en commun le fait que les oxhydryles des carbones 3 et 4 sont opposés par rapport à la chaine (disparition trans), alors que le galactose qui est inactif présente la disparition cis. Cette remarque pour avoir une valeur devrait être vérifiée par l'étude d'autres hexoses.

Quel peut être le mode de métabolisme glucidique chez *T. evansi* ?

Marshall [6] a observé que prennent naissance 1,75 molécule d'acide pyruvique au lieu des 2 théoriques que donnerait la dégradation du glucose selon la première phase du cycle de Krebs. Il conclut que la différence est utilisée par *T. evansi* à la synthèse de ses protéines. Or, si nous versons du sang de rat très parasité dans le milieu synthétique déjà cité, additionné de glucose, on constate très vite l'apparition d'une teinte lie de vin, montrant qu'il y a eu fixation d'une certaine quantité de gaz

Tableau III

Substance	No.	*evansi* normal	*evansi* résist.	*brucei*	Substance	No.	*evansi* normal	*evansi* résist.	*brucei*
Glycogène	1	+++	+++	+	Acide acétiq.	23	–	–	–
Maltose	2	+++	+++	+	Acide citriq.	24	–	–	–
Glucose	3	++++	++++	+++	Acide fumari.	25	–	–	–
Fructose	4	++++	+++	+++	Acide lactique	26	–	–	–
Mannose	5	++++	++++	+++	Acide malique	27	–	–	–
Glycérine	6	+++	++	+++	Acide oxalo. ac.	28	–	–	–
Arabinose	7	–	–	–	Acide succiniq.	29	–	–	–
Ribose	8	–	–	–	Acide tartriq.	30	–	–	–
Xylose	9	–	–	–	Acide α cétoglut.	31	–	–	++
Galactose	10	–	–	–	Pyruvate Na	32	–	–	+
Rhamnose	11	–	–	–	Tréhalose	33	–	–	–
Glucosamine	12	++	++	++	Glycocolle	34	–	–	–
Lactose	13	–	–	–	Alanine	35	–	–	±
Saccharose	14	–	–	–	Serine	36	–	–	–
Raffinose	15	–	–	–	Thréonine	37	–	–	+
Inuline	16	–	–	–	Glutation	38	–	–	–
Dulcitol	17	–	–	–	Acide aspart.	39	–	–	–
Inositol	18	–	–	–	Acide glutam.	40	–	–	+
Lyxose	19	–	–	–	Proline	41	–	–	+
Mannite	20	–	–	–	Ornithine	42	–	–	–
Sorbitol	21	–	–	–	Arginine	43	–	–	–

carbonique sur les hématies ou bien une consommation d'oxygène car une oxygénation par agitation ou par addition d'une eau oxygénée redonne à la suspension sa couleur rouge clair. Ce phénomène est en rapport étroit avec la présence de glucose. Nous en avons d'ailleurs tiré une méthode de mise en évidence dans le sang, de certaines diastases telles que maltase ou glycogénase. La première phase du cycle de Krebs, celle qui aboutit à l'acide pyruvique est anaérobie et nous savons (tableau III) que *T. evansi* n'utilise absolument pas cet acide comme substance énergétique, en outre, nous avons observé qu'en présence de glucose il n'entrave que légèrement le phénomène précédemment décrit. La même observation se répète avec le cyanure de potassium.

Nous avons donc été amenés à penser que *T. evansi* catabolisait simultanément une partie du glucose par un autre processus que le cycle de Krebs, par exemple le cycle gluconique dans lequel on a effectivement dégagement de gaz carbonique et absence de formation d'acide pyruvique. Des recherches dans ce sens doivent être poursuivies.

Un autre aspect curieux du métabolisme glucidique de *T. evansi* est le suivant.

Il semble que du glucose en contact pendant un certain temps avec du sang de rat soit plus rapidement dégradé par le parasite. En effet, si nous réalisons les 2 séries suivantes:

Série I — (effectuée en 2 temps)

a) suspension de sang de rat non parasité en milieu synthétique + solution de glucose +

b) + 24 heures après
suspension en milieu synthétique de sang de rat fortement parasité.

Série II — (effectuée en même temps que la 2e partie de la série I)

Suspension de sang de rat non parasité en milieu synthétique + solution de glucose + suspension de sang de rat fortement parasité en milieu synthétique

on constate que la réduction de l'hémoglobine apparaît toujours en premier lieu dans la série I et ceci bien qu'une certaine glycolyse soit observée.

Pour terminer avec *T. evansi* signalons (tableau III) que la souche résistante à la Lomidine se comporte vis-à-vis des glucides exactement comme la souche normale bien que dans plusieurs expérimentations non citées dans ce travail, le pourcentage de survie ait été très nettement supérieur.

T. brucei (tableau III) présente des différences notables avec *T. evansi.*

La première phase du cycle de Krebs semble moins intense par contre, on constate une mobilisation avec le pyruvate de Na et l'acide α céto glutarique. Il nous a semblé que surtout les formes courtes réagissent avec ces corps.

Les acides aminés suivants: alanine, thréonine, acide glutamique et proline provoquent une mobilisation, donc sont énergétiques, ce sont des glucoformateurs et on est en droit de penser que *T. brucei* est capable d'effectuer cette transformation. Nous éliminons l'action d'une diastase sanguine car dans les mêmes conditions *T. evansi* ne réagit pas. Il est probable que ces glucoformateurs interviennent également par transamination avec l'acide pyruvique.

T. brucei présente donc un système enzymatique plus complet et en quelque sorte mieux équilibré que celui de *T. evansi*, ce qui explique la possibilité de le cultiver.

CONCLUSION

Les deux méthodes utilisées dans ce travail, particulièrement celle que nous avons appelée " test de mobilité ", nous ont permis d'étudier certains points du métabolisme glucidique de *T. evansi* et *T. brucei.*

Les deux souches dont nous disposons sont différenciables biochimiquement par leur comportement vis-à-vis des corps suivants: pyruvate de Na, acide α céto glutarique, alanine, thréonine, acide glutamique et proline. Le test à l'acide α céto glutarique fut le plus net dans nos expériences. Il pourrait servir de base à l'étude de différentes souches de *T. brucei*, particulièrement celles conservées depuis longtemps sur petits animaux et qui ont perdu la faculté de cultiver " in vitro ".

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ELIMINATION DE L'ACIDE PYRUVIQUE DES MILIEUX DE CULTURE EN VUE DE FAVORISER LA SURVIE DE *TRYPANOSOMA EVANSI*

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Trypanosoma evansi, que l'on trouve dans le sang circulant d'un animal trypanosomé, y puise les éléments nécessaires à sa vie et à sa multiplication. Les conditions semblent particulièrement favorables chez le rat où nous avons parfois constaté la présence de plus d'un million de parasites par mm^3 de sang.

On pouvait penser que sa culture " in vitro " serait simple mais pourtant elle n'a jamais été réalisée. On s'aperçoit, en effet que cette culture est sous la dépendance de multiples facteurs, le plus souvent méconnus, et très liés les uns aux autres, c'est-à-dire que la présence ou l'absence de l'un d'eux suffit à rendre impossible toute réussite.

Une des causes d'échec est la formation de déchets et spécialement ceux du catabolisme du glucose, que le flagellé a la possibilité d'éliminer lorsqu'il se multiplie dans le sang circulant.

Kligler, Geiger et Comaroff [5] parlent de l'acide lactique. Christophers et Fulton [4] notent une action nocive de l'acidification sur *T. rhodesiense*. Krijgsman [6] pense que le principal déchet du métabolisme de *T. evansi* est l'acide pyruvique. C'est aussi l'avis de Marshall [7] qui le prouve et trouve par des mesures précises que chez *T. evansi*, les 7/8e du glucose utilisé sont transformés en acide pyruvique qui s'accumule dans le milieu.

Le but de notre travail a été de rechercher en premier lieu quelles étaient l'importance et la rapidité de formation de l'acide pyruvique, puis, après avoir mis en évidence sa toxicité pour *T. evansi* nous avons étudié et expérimenté différents moyens destinés à éliminer cet acide du milieu de culture.

MATERIEL ET METHODES

La souche de *T. evansi* a été prélevée en 1961 sur un âne de Fort-Lamy.

Les différentes expérimentations ont été effectuées soit en milieux liquides, soit en milieux diphasiques:

— Milieux liquides: ils sont de 2 types.

1) Milieu au sang de cheval dont la composition est la suivante

— sang de cheval	10 ml
— liquide " Roche "	1 ml
— eau distillée	90 ml

Hémolyse pendant 1 heure à la température du laboratoire.

— phosphate bipotassique	1 g
— glucose	2 g

— phosphate monopotassique: Q.S. pour avoir pH 7,4.

Filtration sur papier puis sur Seitz.

2) Milieu synthétique de formule suivante

— glucose	2 g
— phosphate bipotassique	1 g
— chlorure de sodium	0,2 g
— eau distillée	100 ml

— phosphate monopotassique: Q.S. pour avoir pH 7,4.

Filtration sur Seitz.

— Milieux diphasiques: ils sont réalisés en tubes à essais avec comme phase liquide l'un des milieux précédemment décrits et comme phase solide de la gélose physiologique à 2 pour cent dans laquelle est incorporée la substance détoxifiante.

Les dosages d'acide pyruvique ont été effectués par la technique de Caron et Raquet [2] modifiée par nous-mêmes.[1]

Toutes les expérimentations ont été réalisées dans une pièce climatisée maintenue automatiquement à la température de 25°.

IMPORTANCE ET RAPIDITE DE FORMATION DE L'ACIDE PYRUVIQUE

Nous avons procédé de la façon suivante: 200 ml de milieu au sang sont ensemencés avec du sang de rat fortement parasité. Toutes les 30 min. on prélève 6 ml du mélange afin de pratiquer un dosage d'acide pyruvique et une numération des trypanosomes.

Le tableau I montre que l'acide pyruvique est nettement décelable dans le milieu dès la première demi-heure. Son taux croît presque linéairement en même temps que la quantité de flagellés diminue. Il semble exister un rapport entre ces deux phénomènes et nous avons été amenés à étudier la toxicité de l'acide pyruvique.

TOXICITE DE L'ACIDE PYRUVIQUE POUR *T. EVANSI*

Cette expérimentation a été faite sur milieux diphasiques avec comme phase solide de la gélose physiologique servant de support à l'acide pyruvique ou au pyruvate de sodium, et comme phase liquide, le milieu synthétique.

Ce dernier est ensemencé en bloc, puis réparti dans les différentes séries de tubes à essais. En début d'expérience, nous avions 40.000 flagellés au mm³.

Le tableau II nous donne les résultats relevés après 20 heures d'incubation.

L'acide pyruvique est nettement plus toxique que son sel de sodium et une

Tableau I

Temps en minutes	Qté de pyruvate en mg par cm³	Numération des trypanosomes
0	0	17.600
30	0,03	17.000
60	0,06	15.600
90	0,09	14.000
120	0,11	12.800
150	0,14	11.000
180	0,16	10.000
210	0,18	8.000

dose de 10 mg pour 120 ml (phase liquide et phase solide) soit 0,08 mg par ml est très nocive. Or, si nous nous reportons au tableau I, nous constatons que ce taux est atteint avant 90 minutes.

Dès la première heure, un milieu liquide peut donc être considéré comme impropre à toute culture de *T. evansi*, même s'il contient les facteurs de croissance nécessaires. Il est donc évident que si l'on veut avoir quelques chances de succès, il faut en premier lieu éliminer l'acide pyruvique du milieu.

Nous avons étudié un certain nombre de procédés (physiques, chimiques et biologiques) qui nous ont permis d'apporter un début de solution à ce problème. Moyens physiques:

Moyens physiques

1) Diffusion dans la gélose

C'est le procédé classiquement employé lorsqu'on cultive les trypanosomes en milieux diphasiques. La phase solide est toujours beaucoup plus volumineuse que la phase liquide et l'acide pyruvique formé diffuse dans la gélose.

Une amélioration du rendement peut être obtenue en diminuant la température d'incubation. En effet, entre 25 et 37° l'intensité du métabolisme de *T. evansi* obéit à la loi de Van't Hoff, c'est-à-dire qu'elle est divisée par 2,5 quand la température baisse de 10°. Par contre, la vitesse de diffusion étant proportionnelle à la température absolue sera, pour le même intervalle, divisée par 1,03. Il est donc possible de diminuer fortement la production d'acide pyruvique tout en ne changeant pratiquement rien à son élimination. Les cultures de trypanosomes pathogènes " in vitro " ne réussissent d'ailleurs qu'aux environs de 25°.

Tableau II

Phase liquide	Phase solide	Nombre de tubes	Nombre de T. par mm³ après 20 h
Milieu synthétique et sang de rat parasité 2 ml par tube	Gélose physiol. 100 ml	10	900
	Gélose physiol. 100 ml + 100 mg d'acide pyruvique	10	0
	Gélose physiol. 100 ml + 10 mg d'acide pyruvique	10	446
	Gélose physiol. 100 ml + 100 mg pyruvate de Na	10	390
	Gélose physiol. 100 ml + 10 mg pyruvate de Na	10	610

2) Diffusion à travers une membrane semi-perméable

Une membrane semi-perméable laisse passer les cristalloïdes et doit donc permettre une élimination correcte de l'acide pyruvique. Afin de vérifier cette hypothèse, nous avons réalisé le dispositif suivant:

Un sac de cellulose contenant 22 ml de milieu au sang (A) ensemencé avec *T. evansi* est suspendu dans une fiole d'Erlenmeyer et baigne dans 133 ml de milieu synthétique (B). 22 ml de milieu au sang, ensemencé, sont gardés comme témoin (C).

Au début de l'expérience, nous avions en A et C, 27.000 flagellés au mm^3. Après 20 heures d'incubation nous avons effectué une numération des trypanosomes sur A et C, puis un dosage d'acide pyruvique sur A, B et C, nous avons noté les résultats suivants:

Numération des trypanosomes:

A: 9.200
C: 15.600

Dosage de l'acide pyruvique:

A: 0,08 mg/ml soit au total $0{,}08 \times 22 = 1{,}76$ mg
B: 0,03 mg/ml soit au total $0{,}03 \times 133 = 4$ mg
C: 0,30 mg/ml soit au total $0{,}30 \times 22 = 6{,}6$ mg

L'acide pyruvique a donc bien diffusé en B mais l'équilibre n'a pas été atteint puisque la quantité au ml est supérieure en A. Par contre, en C l'acide s'est accumulé et le taux en est beaucoup plus important.

Il est certain qu'un dispositif ayant un rapport masse/surface très petit doit permettre d'atteindre des résultats très corrects.

Puisque le principal déchet se trouve en partie éliminé, nous devrions trouver le plus de trypanosomes en A et la quantité d'acide pyruvique contenue en 1 et B devrait être supérieure à celle trouvée en C. Or, c'est l'inverse que nous observons. On est donc amené à penser qu'un facteur favorable a diffusé de A vers B et il est nécessaire d'en tenir compte dans tout système utilisant la dialyse comme moyen détoxifiant.

3) Lavage continu

Cette méthode doit en principe permettre l'élimination totale de l'acide pyruvique. Nous l'avons expérimentée à l'aide du dispositif suivant:

On verse quelques ml de milieu au sang contenant des trypanosomes dans un petit filtre Seitz, puis on ferme sa partie supérieure par un bouchon. Ce dernier est traversé par un compte-gouttes auquel est adapté un tuyau souple amenant du milieu au sang sous une faible pression (40 à 50 cm d'eau). Le liquide tombe goutte à goutte dans le filtre, comprime l'air qui s'y trouve et une fois l'équilibre atteint, il passe à travers le disque d'amiante autant de liquide qu'il en rentre à la partie supérieure.

Les trypanosomes sont donc en suspension dans un milieu continuellement renouvelé. L'expérience montre que l'acide pyruvique est bien éliminé; il n'en reste pas dans le filtre et on n'en trouve que des traces dans le liquide de perfusion. Mais alors qu'au moment de l'ensemencement on dénombrait 4.600 flagellés au mm^3, on n'en retrouve 20 heures après que 1.800 dans le témoin et moins de 10 dans le filtre. Il semble donc y avoir eu privation d'un ou de plusieurs facteurs nécessaires provenant de *T. evansi* lui-même. L'un d'eux est peut-être analogue à l'exoantigène signalé par Weitz [8,9] chez *T. brucei*.

En résumé, la technique du lavage continu permet une élimination très correcte de l'acide pyruvique et des autres déchets de métabolisme mais ne peut

être envisagée qu'avec un milieu contenant tous les facteurs nécessaires y compris ceux provenant de *T. evansi*.

4) Utilisation des propriétés adsorbantes des charbons

Le charbon de bois et le noir animal sont capables à des degrés différents d'adsorber des gaz, des vapeurs et de nombreuses substances minérales ou organiques en solution. Ils se comportent comme une sorte d'éponge présentant une surface interne considérable et leur pouvoir adsorbant est fonction de leur état de division.

Le charbon végétal est loin d'être un carbone pur. Outre quelques gaz, il contient 1 à 8 pour cent de cendres à réaction alcaline.

Le charbon animal purifié possède un pouvoir adsorbant beaucoup plus grand et, en dehors du carbone à l'état très divisé, on ne trouve que des substances sans affinités chimiques comme la silice.

Nous avons constaté que surtout le noir animal purifié était capable d'adsorber de petites quantités d'acide pyruvique.

L'influence des charbons sur la survie de *T. evansi* a été recherchée par la technique des milieux diphasiques; le noir animal est inclus dans la gélose et la phase liquide est du milieu au sang.

Pour 20.000 flagellés au mm^3 en début d'expérience nous avons trouvé après 20 heures d'incubation les résultats suivants:

— témoin: 2.300
— noir animal: 5.600
— charbon végétal: 2.000

L'influence favorable du noir animal paraît assez nette et comme cette substance est chimiquement inactive, seules ses propriétés physiques sont en cause.

Ces résultats s'expliquent si on considère que sont éliminés en priorité les corps de faible poids moléculaire qui diffusent rapidement dans la gélose. Malheureusement, d'autres substances favorables subissent le même sort et les résultats sont différents si on mélange directement le charbon animal au milieu liquide.

En effet, nous avons ensemencé avec la même quantité de *T. evansi* 3 fioles d'Erlenmeyer: A-B-C, contenant:

A: 50 ml de milieu au sang + 1 g de charbon animal
B: 50 ml de milieu au sang traité par 1 g de charbon animal puis filtré sur Seitz
C: 50 ml de milieu au sang

Pour 10.000 trypanosomes au départ de l'expérience, nous avons retrouvé après 20 heures d'incubation:

A: 4.900
B: 6.100
C: 6.700

Le nombre le plus élevé correspond au témoin C et en A, il y a eu adsorbtion d'une partie de l'acide pyruvique mais également de facteurs nécessaires de gros poids moléculaire, provenant du flagellé ou du sang de rat. Ces facteurs se trouvent également dans le milieu au sang, mais en très faible quantité puisqu'un traitement au noir animal suivi de filtration ne modifie que peu la survie.

L'utilisation des charbons ne résoud donc pas correctement le problème, cependant une étude, en fonction du pH, des propriétés adsorbantes d'autres substances peut à notre avis, aboutir à des résultats intéressants.

Moyens chimiques

1) Hydroxylamine

Au cours de diverses expérimentations, nous avons constaté l'effet favorable de substances telles que la Colamine ou l'ammoniaque à très faible dose (1 pour 10.000) et nous avons été amenés à effectuer des essais avec l'hydroxylamine.

Des dosages systématiques de l'acide pyruvique nous ont permis d'observer qu'il était partiellement éliminé par l'hydroxylamine.

Chimiquement, une molécule de sulfate se combine à deux molécules d'acide pyruvique.

Expérimentalement, le sulfate d'hydroxylamine à la dose de 10 mg pour 100 ml de milieu améliore la survie mais présente cependant une certaine toxicité. Cette dernière peut être réduite si on neutralise l'acide sulfurique soit par de la soude, et dans ce cas on obtient un mélange de sulfate de sodium et d'hydroxylamine, soit par traitement au carbonate de baryum en excès et filtration sous vide pour éliminer le gaz carbonique.

Les résultats sont comparables et dans les 2 cas, on peut quadrupler la dose d'hydroxylamine. Mais la toxicité du composé formé avec l'acide pyruvique, n'étant pas négligeable, ce procédé n'offre que des possibilités limitées.

2) Sulfite et bisulfite de sodium

Ces deux corps semblent se combiner à l'acide pyruvique dans des proportions qui ne sont pas définies.

Leur toxicité est nettement moins importante que celle de l'hydroxylamine puisqu'on peut en ajouter 100 mg pour 100 ml de milieu.

Leur action favorable sur la survie est surtout due, à notre avis, à leurs propriétés réductrices car des corps tels que la cystéine, la glutathion réduit ou la réductone ont le même effet.

Moyens biologiques

L'étude de ces moyens relativement complexes, n'a été qu'ébauchée.

De nombreux micro-organismes sont capables de proliférer dans un milieu synthétique, ne contenant que de l'azote minéral et du pyruvate de sodium. Ils élaborent rapidement de l'alanine et de l'acide glutamique. C'est le cas des germes suivants:

Proteus vulgaris, *Pseudomonas aeruginosa*, *Salmonella paratyphi B* (Césaire-Boiron-Kerharo et Attiso [3]).

D'autres germes sont complètement autotrophes et peuvent faire la synthèse de leurs acides aminés à partir de l'azote atmosphérique et de l'acide pyruvique. C'est le cas de l'Azotobacter présent dans le sol et que l'on isole facilement sur terre moulée additionnée de pyruvate de sodium.

Des essais de culture en symbiose avec *T. evansi* n'ont pas été concluants car l'Azotobacter n'a pas une croissance très rapide et n'absorbe de ce fait qu'une faible partie de l'acide pyruvique.

De plus, cultivé dans un milieu contenant du glucose il utilise de préférence ce dernier et on ne retire donc aucun bénéfice de l'association.

A notre avis, il y a des chances de succès en utilisant un germe à croissance rapide tel que *Proteus vulgaris* à partir duquel on pourrait opérer une sélection sur milieu synthétique au pyruvate. En extrayant des corps microbiens les enzymes présidant au catabolisme de l'acide pyruvique et en les ajoutant au milieu au sang, on complèterait en quelque sorte l'appareil enzymatique de *T. evansi*.

CONCLUSION

L'accumulation d'acide pyruvique dans le milieu constitue un obstacle majeur à la culture " in vitro " de *T. evansi*. Il est donc nécessaire d'éliminer ce déchet de métabolisme et différents moyens d'ordre physique, chimique et biologique ont été expérimentés.

Dans l'état actuel de nos recherches, les moyens physiques nous semblent les plus efficaces et ont le grand avantage d'être dépourvus de toxicité. Cependant, ils permettent également l'élimination de facteurs non identifiés indispensables à la multiplication de *T. evansi* et on doit tenir compte de ce fait dans les réalisations techniques.

Une détoxication chimique est assez délicate à manier car en définitive on recule le problème en remplaçant une substance toxique par une autre qui l'est un peu moins.

Enfin, les techniques biologiques bien que théoriquement pleines de promesses nécessitent encore de longues recherches avant d'aboutir au résultat désiré.

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THE DURATION OF INFECTIVITY TO MICE OF TRYPANOSOMES INGESTED BY *GLOSSINA PALLIDIPES*

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During recent (1961) epidemiological investigations in Central Nyanza, Kenya, it was necessary to isolate trypanosomes from wild-caught *Glossina*. The procedure was as follows. Groups of flies (ten or less) were triturated with powdered glass in phosphate buffered saline, pH 8, at 0° C. After standing for thirty minutes at 0° C., the supernate was inoculated into mice; the trypanosome isolates obtained were preserved at approximately —80° C. Since it was thought possible that these isolates might be derived from organisms freshly ingested by the flies, and thus that a positive isolation need not necessarily indicate that a fly was capable of transmitting infection, it was decided to determine the length of time that trypanosomes ingested by *Glossina* remained infective to mice.

G. pallidipes were fed on a capsule containing defibrinated bovine blood to which was added heparinised blood from a rat infected with a strain of the *T. brucei* Subgroup isolated from man (probably *T. rhodesiense*). At three hours, and at twenty-four-hour intervals up to ninety-six hours after feeding, ten flies were triturated in buffered saline with glass powder for forty-five seconds. The resulting suspensions were diluted serially tenfold and inoculated into groups of five mice. As a comparison, 0·376 ml. (equivalent to the volume taken up by ten flies) of the blood mixture on which the flies had fed was treated in the same way at the same time intervals. Trypanosome counts were also made of each material after each interval, using a haemacytometer. The results are shown below where the figures are expressed as logarithms (base 10).

Interval after feeding (hours)	Triturated flies		Triturated blood pool	
	ID_{50} per ml.	Trypanosomes per ml.	ID_{50} per ml.	Trypanosomes per ml.
0	—	—	—	6·1*
3	4·50	5·5	5·2	6·0
24	<1·5	5·3	3·7	5·8
48	<1·5	4·7	2·5	5·9
72	<1·5	None seen	<1·5	5·2
96	<1·5	None seen	<1·5	4·8

*Not triturated.
Numbers of ID_{50}'s and trypanosomes are expressed as logarithms to the base 10.
<1·5 indicates no mice infected in the 1 log. dilution, the lowest inoculates.

The trypanosomes in the blood ingested by the flies were infective to mice at three hours but not at twenty-four hours, whereas those in the blood pool, which had been kept at similar temperatures in the laboratory, were infective up to

forty-eight hours. The corresponding trypanosome counts show that live trypanosomes were found in both the gut of the flies and the blood pool for about forty-eight hours after they had ceased to be infective to mice.

Using the method described in the introduction, three *T. brucei* Subgroup isolates were obtained from 1,068 *G. pallidipes* None of these flies had obviously recently fed when captured, and were triturated at least twenty-four hours after capture. On inoculation into man, a positive parasitaemia was obtained following a prepatent period of eleven days. It was concluded from these results that *G. pallidipes* is a vector of human trypanosomiasis in Central Nyanza District, Kenya (Lumsden *et al.*, 1963).

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SEPARATION OF TRYPANOSOMES FROM RAT BLOOD COMPONENTS

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A method for obtaining as nearly as possible 100% yield of trypanosomes free from rat red and white corpuscles and platelets was considered essential in order to study the interspecific differences in trypanosome protein.

Infected blood from rats in the second or third day of parasitaemia was collected from the heart into a sterile heparinised syringe. To each 1 ml. blood 5 ml. phosphate buffered salts solution at pH 7·8 containing 1% glucose (B.S.G.) was added, then 1 ml. ox plasma was added to every 10 ml. of the diluted blood to agglutinate the erythrocytes. Ox plasma was used at this stage in preference to rabbit antiserum as it was more readily available, before use it was checked that it was non-trypanocidal and titrated against whole infected rat blood. After mixing, the suspension was left in an incubator at 25° C. for thirty minutes for agglutination to take place.

The suspension was centrifuged lightly at 0–4° C. by taking up to 1,000 r.p.m. in thirty seconds, maintaining at that speed for a further thirty seconds and then switching off (an M.S.E. Magnum Refrigerated Centrifuge with head No. 6876 was used throughout). The deposited red blood corpuscles were washed three times by mixing with 40 ml. fresh B.S.G. and repeating the centrifugation process. All supernatant fluids were pooled and a check made by counting RBC, trypanosomes and WBC using a haemocytometer to ensure that over 75% of the trypanosomes remained. If less, then the deposit was rewashed. The suspension was then centrifuged at 2,700 r.p.m. for fifteen minutes at 0–4° C. and the resulting precipitate (consisting mainly of trypanosomes together with some agglutinated RBC and WBC) made up to 40 ml. with B.S.G. then 2 ml. rabbit anti-rat red and white corpuscle serum added to agglutinate the remaining rat blood cells. This anti-serum was prepared by inoculating rabbit i.v. twice weekly with 1 ml. of a 50% suspension of washed rat RBC and WBC, and used when the titre was sufficiently high, usually after the fourth to sixth inoculation. Ox plasma was unsuitable at this stage as it did not appear to agglutinate white cells and, unless well diluted, formed a fibrin clot.

After being thoroughly mixed, the suspension was left at 25° C. until agglutination occurred, usually in ten to fifteen minutes, then centrifuged by taking up to 1,000 r.p.m. at 0–4° C. in thirty seconds and switching off. The agglutinated cells were washed three times as before with B.S.G. to remove any trapped trypanosomes; the supernates were then pooled and centrifuged for fifteen minutes at 2,700 r.p.m. at 0–4° C. This final deposit consisted almost entirely of trypanosomes and a count was made using a haemocytometer to check the number of remaining trypanosomes, WBC and RBC. Three further washes of trypanosomes by centrifugation at 2,700 r.p.m. ten minutes 0–4° C. in B.S.G. were carried out and each time the supernatant was treated with 3% sulphosalicylic acid to detect traces of plasma or serum protein. If protein was present, the washing process was repeated.

The final trypanosome deposit was either stored, or used immediately for electrophoretic investigations of constituent proteins. At all stages during the extraction, haemocytometer counts were carried out. Counts were performed in duplicate and for reproducibility, 400 cells or trypanosomes were counted, or if fewer, the number in nine large squares of an improved Neubauer chamber. No suitable method for counting platelets was devised, they were detectable in whole blood but after the first agglutination stage none were found in stained smears.

In a typical separation, the total number of trypanosomes, RBC and WBC in 58 ml. of blood were $26{\cdot}6 \times 10^8$, 1950×10^8 and $0{\cdot}26 \times 10^8$ respectively. After the addition of ox plasma and washing, the numbers of trypanosomes and RBC were $26{\cdot}4 \times 10^8$ and 132×10^8 respectively, no WBC were counted as most were removed by agglutination. Counts on the final preparation of trypanosomes showed that 25×10^8 trypanosomes were still present but the numbers of RBC were reduced to $0{\cdot}25 \times 10^8$, i.e. one RBC to every 100 trypanosomes. The trypanosome material remained infective throughout the separation as 0·1 ml. inoculated into a clean rat at each stage subsequently produced an infection.

For storage, the trypanosome deposit was suspended in an equal volume of distilled water, frozen and thawed ten times, then freeze dried. Material disrupted by using the Mickle disintegrator tended to yield poor results on electrophoresis owing to protein denaturation. Solutions obtained from disrupted material produced by ultrasonic disintegration contained much soluble protein, but after storage at −80° C., much of the protein material appeared to have precipitated. So far, the best results obtained by electrophoretic separation of the trypanosome proteins have been from freshly isolated material. (Possibly disrupted material stored at 4° C. would retain its potency longer than that preserved at lower temperatures.) For electrophoretic runs being carried out at present, the fresh trypanosome material was frozen and thawed ten times in plain microhaematocrit tubes then spun down in a microhaematocrit centrifuge for two minutes. The supernatant was retained and concentrated to one-tenth of its volume with Sephadex G25 and this was then applied direct to the gel or membrane. The separation described normally took about three hours to complete.

If a more rapid trypanosome separation was required with less attention being paid to yield and purity, whole infected rat blood diluted 1 + 3 B.S.G. was treated with rabbit anti-serum 1 ml. to 10 ml. diluted blood and left to agglutinate. The resulting supernatant fluid containing trypanosomes was syphoned off and the trypanosomes spun down for ten minutes at No. 10 in an M.S.E. minor centrifuge. This process took about thirty minutes for completion.

The longer method of separation has been found successful with *T. brucei*, *T. rhodesiense* and *T. vivax*. *T. gambiense* was also isolated but yields were poor owing to the low parasitaemias in rats, while the yield of *T. congolense* tended to be low as the trypanosomes adhered to the red blood corpuscles and were trapped during agglutination.

SECTION II

ENTOMOLOGY/ENTOMOLOGIE

LONG-TERM FLUCTUATIONS IN NUMBERS OF A POPULATION OF *GLOSSINA PALPALIS PALPALIS* (R.-D.): SIXTEEN YEARS' OBSERVATIONS

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A population of *G. palpalis palpalis* (R.-D.), near Kaduna in Northern Nigeria, was intensively studied over a period of six years (1944–50); the results were reported by Nash and Page (1953). In April 1954 some of the fly rounds carried out during the detailed investigations of Nash and Page were resumed and are being continued, in order to investigate whether any regular long-term fluctuations in the numbers of *G. palpalis* can be detected. Detailed analysis of the data will have to await at least a further ten years' results and the object of this paper is simply to present the results obtained from the first ten years of continuous observations and to discuss briefly their significance in relation to other available data on this and other populations of *Glossina*.

It is believed that these investigations represent the longest continuous period of comparable studies that have been undertaken on any species of riverine tsetse and that only the long-term studies on the savannah tsetse, *G. swynnertoni* Aust., described by Glasgow and Welch (1962) and now discontinued, have been of greater duration. These latter investigations were carried out near Shinyanga, Tanganyika, and were based on regular fly-round observations over twenty-three years. There has been a number of other shorter studies on tsetse populations, including an investigation, of just over six years' duration, into the ecology of *G. fuscipes fuscipes* Newst. (formerly considered as *G. palpalis fuscipes* Newst.) near Lake Victoria (Glasgow, 1954).

DESCRIPTION OF THE INVESTIGATION

The population of *G. palpalis* that was studied inhabited fringing forest along the River Bahago, some fourteen miles NNE of Kaduna, in the Northern Guinea Savannah vegetation zone (Keay, 1953) of Nigeria. The stream passed through an uninhabited forest reserve and the vegetation remained undisturbed by human activity throughout the investigation. A detailed description of the area is already available (Nash and Page, 1953). Nash and Page divided the stream into sections, designated by various colours, along which regular fly rounds were carried out. When investigations were resumed in 1954, catching was carried out only along the main fly round (" Red, White and Blue sections ") of Nash and Page. This fly round, which tapped 1,800 yards of fringing forest, was divided into 100-yard-long sub-sections and one day's catching lasted approximately three hours forty minutes. Catches were made twice a month, the fly round being carried out in alternate directions each fortnight (catches were carried out four times a month during the more detailed observations of Nash and Page). The catching team consisted of two catchers and one recorder. All flies caught were released at the end of each day's work.

The climate in the vicinity of the River Bahago has been described in detail by Nash and Page, and no meteorological data have been collected since the termination of their investigation.

Occasional specimens of both *G. tachinoides* Westw. and *G. morsitans submorsitans* Newst. were caught during this study, but the fringing forest of the stream was pre-eminently a habitat of *G. palpalis*.

RESULTS

In their detailed study, Nash and Page (1953) described numerous aspects of the ecology of *G. palpalis* in Northern Nigeria. In addition to fly rounds (from which data were obtained on such subjects as abundance, sex ratio and sectional preferences of the flies throughout the year), fly marking experiments, regular puparia rounds and the keeping of flies in tubes in different parts of the stream bed were undertaken. In the subsequent investigations described here only one regular fly round was carried out and the results refer to fluctuations in the *G. palpalis* population of the years of the investigation. Preferences shown by the flies for particular sections of the fly round have not been studied in detail; no marked differences from the seasonal pattern described by Nash and Page (*loc. cit.*) have been detected.

In Fig. 1 the monthly fly density along the fly round is expressed in terms of flies per catcher per 100 yards. The sex ratio of the flies caught in each month is also given, together with the monthly rainfall recorded at the West African Institute for Trypanosomiasis Research, some fourteen miles away. The rainfall data obviously do not refer to the precise amount of rain that fell on the fly round, but the patterns of seasonal variations in rainfall in the two localities were the same. Fig. 1 also shows comparable data, abstracted from Nash and Page (1953), for the years 1944–50; rainfall measurements were then made near the fly round.

During their investigation, Nash and Page found that 49% of the 17,306 flies caught were females. From 1954–64, 43% of the 10,107 flies caught were females. The variation from one month to another was greater during the latter period; this was probably mainly because of the smaller size of sample (two fly rounds per month compared to four per month in 1944–50).

The seasonal variations in fly density between 1954 and 1964 confirmed the situation described by Nash and Page (*loc. cit.*). Over the sixteen years for which data are available, there was generally a wet season period of increase, resulting in a peak population at the end of the rains; in six seasons this peak occurred in August, in seven in September, two in October and one in November. The population then declined to reach its lowest level at the end of the dry season or early in the rains, generally in May. The difference between maximum and minimum populations in any one year varied; the greatest difference occurred in 1944–5, when the maximum population exceeded the minimum by a factor of 12·9. From 1959 to the end of the period under consideration, annual variations in fly density were greatly reduced; in 1963–4 the maximum population exceeded the minimum by a factor of only 2·3.

Fig. 2 shows the percentage of female flies in each year's catch, the annual rainfall and the mean fly density for each year. The latter values were calculated by taking the geometric mean of the twelve monthly values (in terms of flies per catcher per 100 yards) for each year of observation. It should be noted (see Fig. 1)

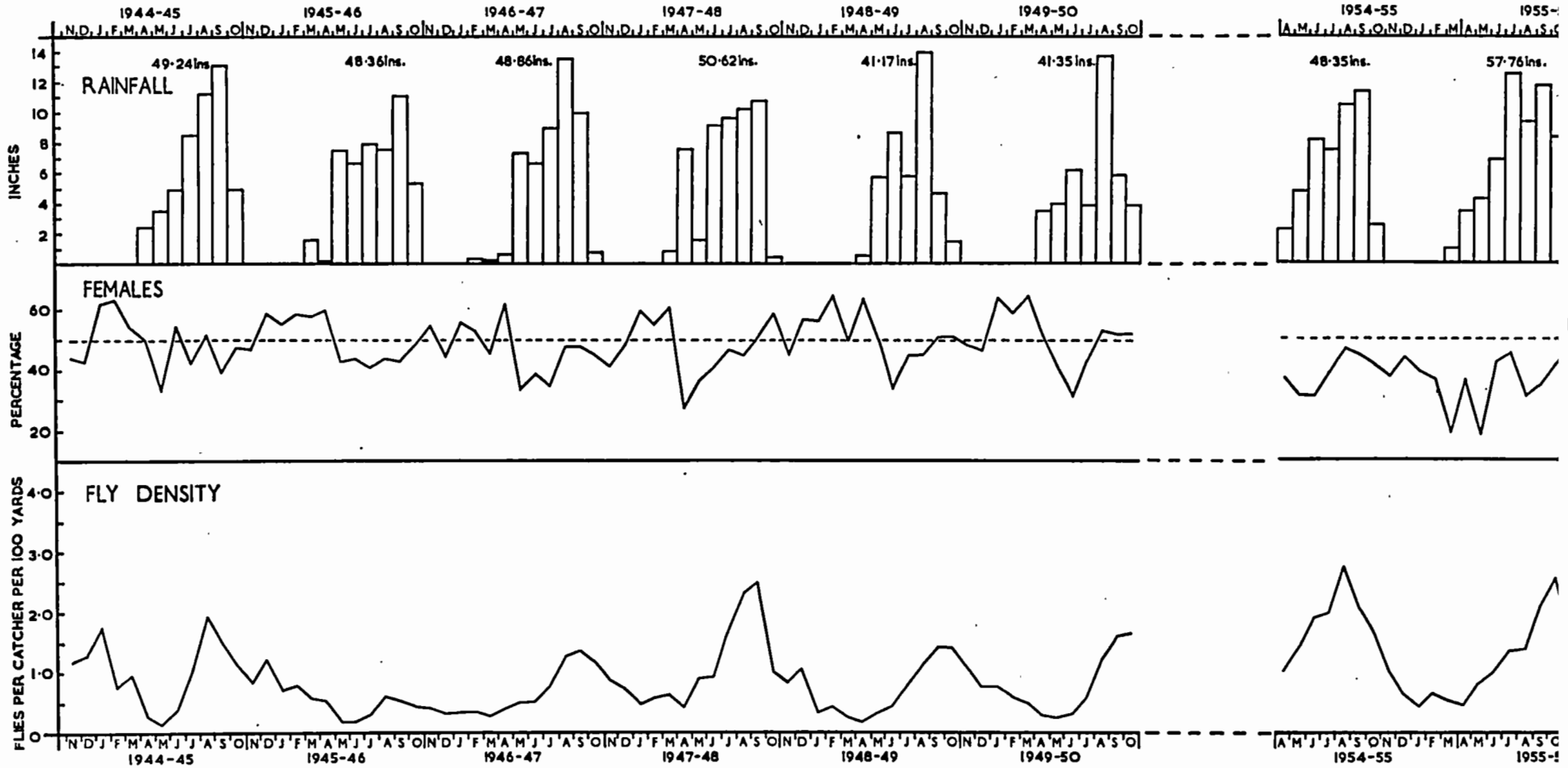
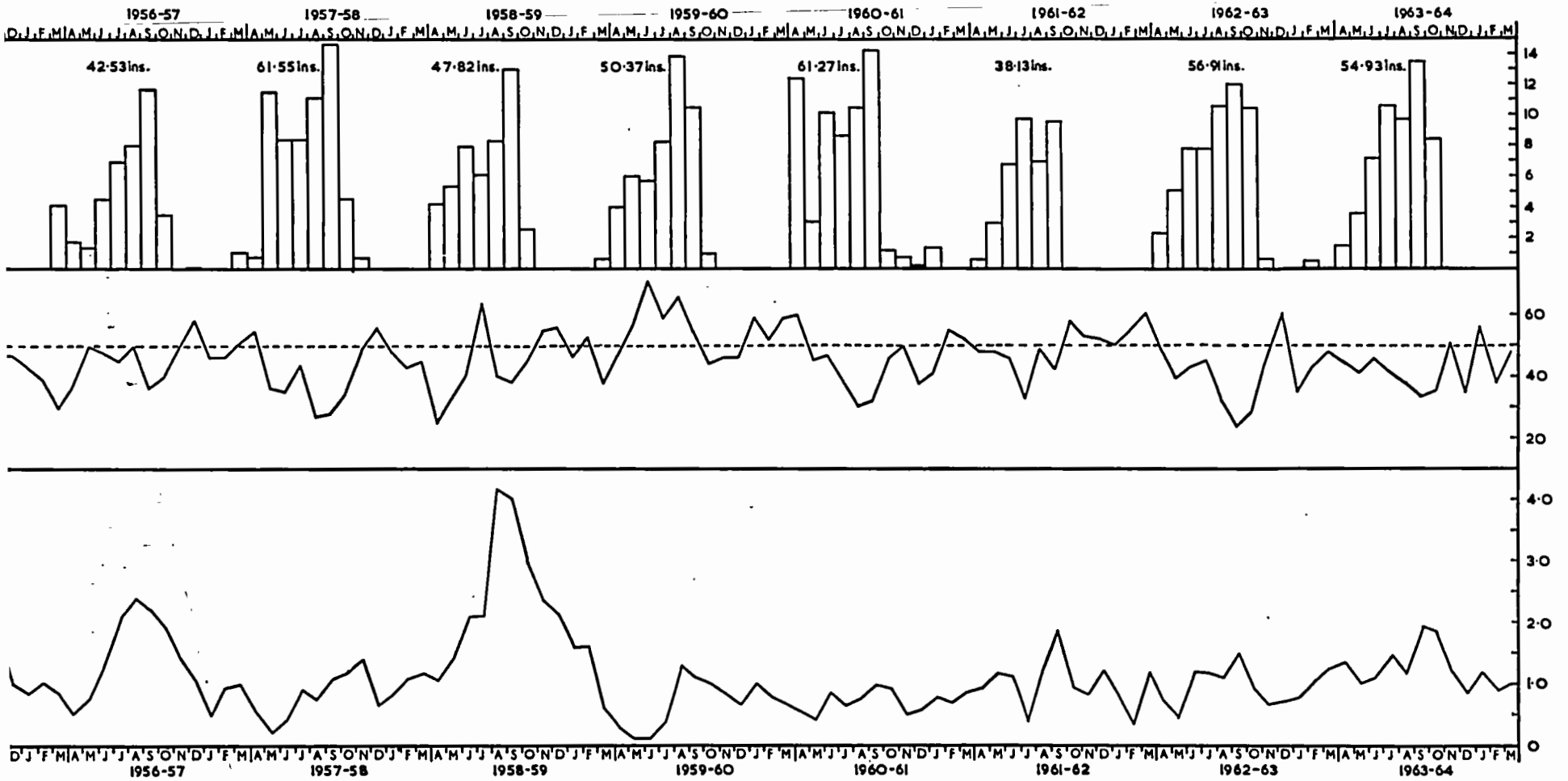


FIG. 1.—Monthly fluctuations in the density of *G. palpalis*, the pe



ntage of females in the total monthly catch and monthly rainfall.

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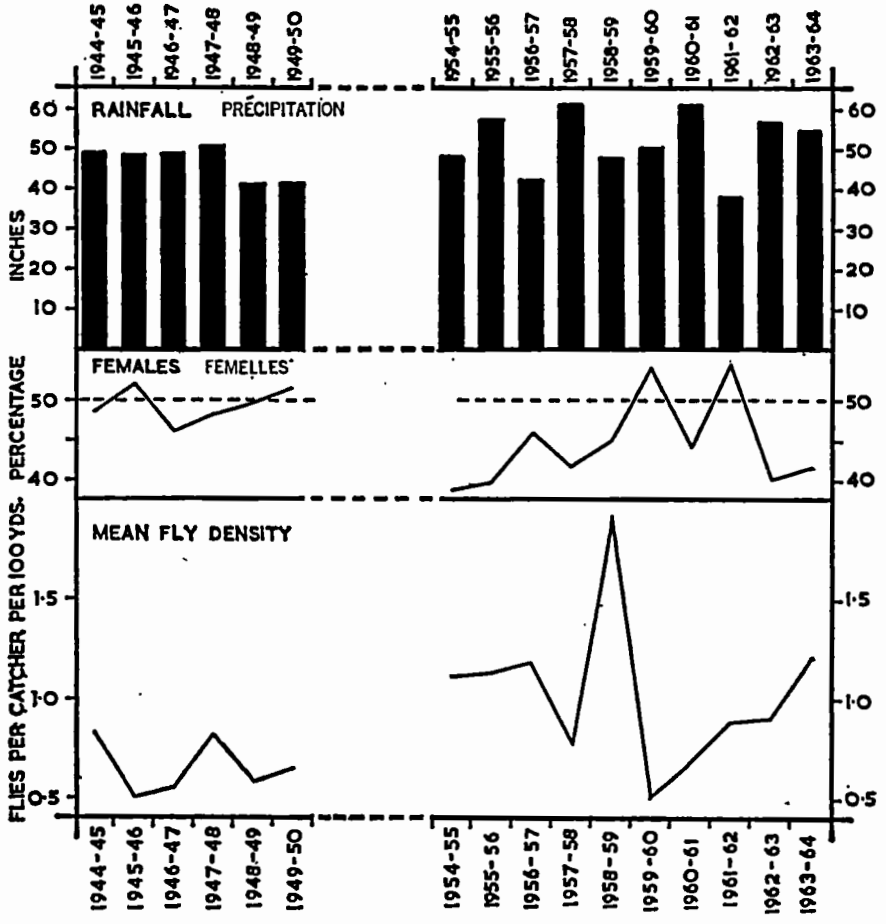

FIG. 2.—The mean annual density of *G. palpalis*, the percentage of females in the total annual catch and annual rainfall.

that in 1944–50 a year ran from November to October and in 1954–64 from April to March. The data show that the mean density of *G. palpalis* varied little in the sixteen years in which observations were carried out and that, so far at least, no regular periodicity in numbers can be detected. Fly density remained remarkably constant from one year to another and the peak value (1958–9) was only 3·8 times the minimum value (1945–6). Conditions were particularly favourable for the fly in 1958–9, and if this exceptional year is omitted the next highest year (1963–4) exceeded the minimum value by a factor of only 2·4.

DISCUSSION

The fringing forest of the River Bahago is an especially suitable environment in which to study long-term fluctuations in numbers of a species of *Glossina*. As the area is a forest reserve and uninhabited, human disturbance of the habitat has been negligible and is not likely to increase in the foreseeable future. Annual burning of

the savannah grasses on either side of the stream takes place, but the fires do not penetrate into the riverine vegetation, the habitat of *G. palpalis*. The only other investigation into long-term fluctuations in numbers of a tsetse population, as far as the author is aware, was carried out in Block 9, Shinyanga, Tanganyika, where a population of *G. swynnertoni* was studied (Glasgow and Welch, 1962). Here the environment was not as stable as the River Bahago as " . . . part of (Block 9), and possibly all of it, has been maturing, in the vegetational sense, during the period of our observations ". There was also a varied policy concerning the exclusion of fire from Block 9. It seems possible that the gradual maturing of the vegetation in Block 9 could have been at least partly responsible for the general tendency for the population of *G. swynnertoni* to increase between 1935 and 1957 (Fig. 2, Glasgow and Welch, *loc. cit.*). However, although it is considered that the River Bahago environment was much more stable than that at Shinyanga, this advantage was to some extent offset by the much larger samples that were taken at Shinyanga. The fly rounds in Block 9 totalled some thirty miles, compared to the 1,800 yards at the River Bahago. As a population of a savannah tsetse was being sampled at Shinyanga extensive and intricate fly rounds were possible, but as a population of a riverine tsetse was being sampled at the River Bahago thirty miles of fly rounds were quite impracticable. Because of the limitation of the flies to the shade afforded by the riverine vegetation, the fly rounds had of necessity to be linear, following the watercourse.

It has been shown that, in any one year, the maximum population of *G. palpalis* exceeded the minimum by a factor varying from 2·3 to 12·9. The annual mean fly density varied little from year to year; for the sixteen years for which data are available the peak value was only 3·8 times the minimum value. Glasgow and Welch (1962) found that, during twenty-three years, the peak mean annual population of *G. swynnertoni* exceeded the minimum by a factor of 18. In a subsequent discussion of the data and references to various studies on other species of animal, Glasgow and Welch (*loc. cit.*) concluded that fluctuations of this magnitude were small compared to those that occur in other animal populations. The magnitude of the fluctuations so far observed at the River Bahago would thus appear to be remarkably small and it is considered that the relative stability of the fly population there is the most important fact that has so far been obtained in this investigation. It is considered that the greater stability of the population of *G. palpalis* by the River Bahago compared to the population of *G. swynnertoni* at Shinyanga can be related to two main factors. Firstly, the much more stable environment at the River Bahago, where human disturbance has been negligible. Secondly, the habitats of the two species of tsetse are very different; the insulated riverine environment of *G. palpalis* is much less affected by monthly and year-to-year weather changes than the open savannah environment of *G. swynnertoni*, which varies tremendously in these respects and also in, for instance, the effectiveness of the annual burning of the bush. Analysis of data obtained by Glasgow (1954) during a study of the riverine tsetse *G. fuscipes fuscipes* (then considered as *G. palpalis fuscipes*) confirms that populations of riverine tsetse probably fluctuate less than populations of savannah tsetse; the maximum monthly apparent density of the most variable population studied exceeded the minimum population by a factor of only 7·3. No evidence of regular annual fluctuations of fly numbers was found during this investigation.

Both Fig. 1 and Fig. 2 show that there was an exceptionally high density of

G. palpalis by the River Bahago in 1958–9, particularly in August–September 1958. Nash and Page (1953) demonstrated that, in general, the greatest decrease in the *G. palpalis* population occurred in January–April (mid-dry season to start of rains), associated with greatly reduced fly longevity (caused by high maximum temperatures and saturation deficits) and a reduced reproduction rate (caused by low mean temperatures in November, December and January). They also showed that the period of maximum increase of the population was May–August (early to heavy rains) associated with maximal longevity (no dangerously high maximum temperatures and the saturation deficit favoured long life) and maximal rate of reproduction (caused by the very high mean temperatures of March, April, May and June). Fig. 1 shows that during the period of rapid population increase in 1958 the population increased by 3·9 times from April to August, which was no greater than in other years (the increase from May to August in 1945 was 12·9 times). Thus it seems that the high peak of 1958 was not caused by a more spectacular increase in numbers than usual, but rather by the failure of the preceding unfavourable season (January–April 1958) to reduce the population to the low level usual at that time of the year. A more marked failure occurred in 1963, but it seems that the subsequent optimum season was not sufficiently favourable to allow the population to attain a high peak later in the year. It appears, then, that really high populations would be the result of a mild unfavourable season followed by a combination of conditions that allow a rapid period of increase. No meteorological measurements were made at the River Bahago from 1954 onwards and measurements taken at Kaduna, some fourteen miles away, in a Stevenson Screen, fail to indicate whether or not the January–April period in 1958 was demonstrably less critical for *G. palpalis* than in other years.

It is hoped that these investigations can be continued indefinitely; after the accumulation of much more data these preliminary findings may well have to be modified.

SUMMARY

(1) Fly rounds have been carried out for ten years (1954–64), following a previous period of six years' (1944–50) more intensive studies, in an attempt to determine whether or not long-term fluctuations occur in the numbers of a population of *G. palpalis palpalis* (R.-D.) by a stream near Kaduna, in Northern Nigeria.

(2) The greatest difference between maximum and minimum monthly populations in any one year varied from a factor of 12·9 in 1944–5 to one of only 2·3 in 1963–4.

(3) The mean fly density for each year was calculated by taking the geometric mean of the twelve monthly values for each year of observations. Density remained remarkably constant from one year to another and the peak value (1958–9) was only 3·8 times the minimum value (1945–6). It is concluded that, to date, the population of *G. palpalis* has been remarkably stable and that no regular periodicity in mean annual fly density can be detected.

(4) The results obtained for *G. palpalis* near Kaduna are compared with those obtained during a longer investigation on long-term fluctuations in numbers of *G. swynnertoni* Aust. in Tanganyika. In the twenty-three years of the latter study, the peak mean annual population of *G. swynnertoni* exceeded the minimum by a factor of 18. This is considered to be much lower than is typical of many animal species; it is concluded that the even lower value observed for *G. palpalis* near

Kaduna might be expected from differences between the savannah and riverine habitats of the two species of tsetse and the extent of human interference (negligible in the *G. palpalis* habitat) in the two experimental areas.

(5) The density of the population of *G. palpalis* was much higher in 1958–9 than in other years; it is suggested that this was not caused by a more spectacular increase in numbers compared to other years, but rather by the failure of the preceding unfavourable season to reduce the population to the low level usual at that time of the year.

Acknowledgements

I am greatly indebted to the staff of the Entomology Section of the Institute, under the very able supervision of Alhaja A. Da'u, M.B.E., who have carried out the fly rounds throughout this investigation.

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SEASONAL VARIATIONS IN AGE AND INFECTION RATE OF *GLOSSINA FUSCIPES*

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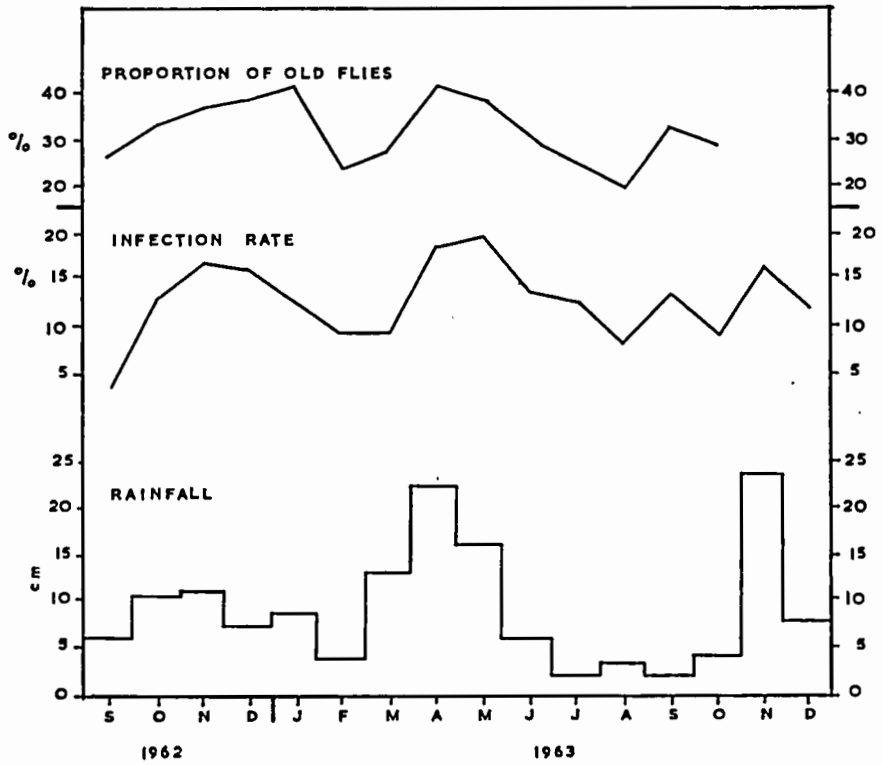
Seasonal variations in infection rate of wild-caught *Glossina* were first recorded in Nigeria (Lloyd *et al.*, 1924) and have since been noted in a number of species. In the majority of cases the infection rate was found to be greatest during the rains and lowest during the dry season. It was at first considered in West Africa that this was due to cessation of breeding during the rains (Lloyd *et al.*, 1924) resulting in a rise in mean age of the population but, although Nash (1936) showed that breeding did not stop there during the rains, both he and a number of other authors consider that there is, in general, a rise in mean age of tsetse populations at this season. However, in the only attempt to correlate age of *Glossina* with infection rate it was found that infections of *G. palpalis* in each of the age groups recognised increased in the wet season and decreased in the dry, and that there were no corresponding seasonal changes in mean age of the population (Squire, 1951; 1954). The conclusion about the mean age has not been generally accepted as applicable in all areas and recent work in Uganda on female *G. fuscipes*, a species closely related to *G. palpalis*, has shown that it does not apply there.

Female *G. fuscipes* were collected at Lugala, Uganda, on the north-eastern shore of Lake Victoria, during twenty-four hour catches on bait cattle. A total of 2,564 were dissected during the fourteen months from September 1962 to October 1963, the number per month varying between 103 and 237. The mean infection rate with all trypanosome species, but excluding infections of the gut only, was 12·6%. The same flies were also dissected to determine their physiological age (Saunders, 1960) and were separated into five age categories depending on the number of times they had ovulated. The first four categories are probably each of about twelve days' duration, so that flies in the fifth and oldest category are more than about fifty days old. Flies in this latter category are otherwise of unknown age and could be 100 days old or more. Of the total catch over the fourteen months, 49·8% were in the youngest category and 31·4% in the oldest. Thus there were relatively few flies in the three intermediate categories.

The percentage of flies in the oldest age category, i.e. those over approximately 50 days old, was taken as a measure of the age of the sample collected each month and was compared with its infection rate. The results, together with rainfall data, are shown graphically in the figure where data for infection rate and rainfall are also included for November and December 1963, a time when no age dissections were carried out.

There was a marked increase in infection rate during the rains and a corresponding decrease during the dry seasons. The infection rate was at a maximum at about the end of the rains and at a minimum at about the end of the dry weather. These fluctuations in infection rate were closely associated with similar fluctuations in age, as measured by the percentage of old flies in the samples, and there was a positive correlation between the two with a coefficient of 0·82, which is highly significant ($P < 0.001$). This degree of correlation is remarkable when it is considered that other sources of possible seasonal variation in infection rate, such as



temperature and differences in the host species available, are not taken into consideration.

Thus at Lugala, seasonal fluctuations in the infection rate were closely correlated with changes in age of the samples, the converse of Squire's (1951, 1954) findings with *G. palpalis* in Sierra Leone. Though mean age was higher during the rains and lower during the dry seasons, there was no cessation of breeding during the rains as very young flies were still abundant then. A rise in mean age should result in an increase in population which would be expected to be reflected in the size of the catch. There was, however, no increase in the numbers collected on the bait cattle during the rains, with the exception of a slight increase in December, and there was certainly no large increase in April or May, during the main wet season, but this may have been the result of lower activity owing to cloud and rain.

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INVESTIGATIONS OF THE STERILE MALE TECHNIQUE WITH *GLOSSINA MORSITANS*

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Utilisation of the sterile male technique for control of tsetse flies (*Glossina* spp.) depends upon ability to release sufficient numbers of vigorous sterile males into natural populations or to sterilise the males in those populations. Investigations with gamma radiation (Potts, 1958) and with chemosterilants (Chadwick, 1964) indicated that the reproductive potential of *G. morsitans* could be reduced by sexual sterilisation. Following the philosophy set forth by Knipling (1963), an intensive study of both sterilisation and mass-rearing of *G. morsitans* has been initiated in Africa (Smith and Dame, 1963). Investigations are being conducted jointly by the United States Department of Agriculture and the Agricultural Research Council of Central Africa under the auspices of the Agency for International Development and the International Atomic Energy Agency. These programmes have been active for less than a year. Thus, information summarised in this progress report must be regarded as preliminary.

STERILISATION STUDIES

For laboratory studies of the sterilisation of *G. morsitans*, field-collected pupae from the Zambesi River valley are currently being utilised. Emerging adults are sexed daily to ensure virginity of experimental stock. Sterilisation treatments are evaluated by confining mature males (six days of age or older) together with mature females (two days of age or older) for a reproductive period of four weeks. Twenty-five pair of insects are housed in a cage measuring 8 × 8 × 11 inches. Insects are fed for one hour each day on guinea pigs placed within a wire-mesh mould inserted into one side of the cage. During non-feeding periods, the cage is held over a tray of sand. The deposited larvae drop into this collection tray through wide-mesh netting that covers the bottom of the cage. At the end of the evaluation period, pupae are gathered from the sand and held for adult eclosion. The number of pupae and resulting adults produced represents the major criterion in the assessment of sterility. In these tests insemination of females usually exceeded 95% but occasionally dropped to a minimum of 80%. An atmosphere of 79° ± 2° F. and 60–70% relative humidity was maintained during these tests.

Chemosterilants were applied by allowing two-day old males to rest for four hours on residual deposits of 10 mg. of tepa, metepa, or apholate/sq. ft. of glass.[3] Even deposits on glass were obtained by pipetting 5 ml. of sterilant-methanol solution into a jar and rotating the jar until dry.

In another series, pupae were dipped for 60 seconds in 50% aqueous methanol solutions containing 5% tepa, metepa, or apholate. Radiosterilisation investigations have been primarily confined to irradiation of pupae; in one instance, however,

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[3] tepa = tris(1-aziridinyl)phosphine oxide; metepa = tris(2-methyl-1-aziridinyl) = phosphine oxide; apholate = 2,2,4,4,6,6-hexakis(1-aziridinyl)-2,2,4,4,6,6-hexahydro-1,3,5,2 = 4,6-triazatriphosphorine.

adults which emerged during the irradiation period were classified as irradiated adults and tested separately. By utilising a cobalt-60 radiotherapy gamma source, pupae were exposed to dosages between 1 and 12 krads at intervals of 0·5 or 1·0 krad. Pupae were held in fine-mesh gauze bags in a ventilated plastic box (15 × 15 cm.) clamped 55 cm. below the source. Dose rates of 54 and 121 rads/min. were used. Variation across the field was 5% and backscatter comprised less than 1% of the primary radiation. Results are presented in Table I. In the adult residual treatment tepa caused complete sterility, metepa gave high sterility, and apholate gave inconsistent results producing moderate sterility. Both tepa and metepa produced complete sterility in the pupal dip treatments, but apholate was ineffective. Although males treated on residual deposits of tepa and metepa apparently competed equally with untreated males, their longevity was reduced: 50% mortality occurred after thirty-five days for tepa-treated males, forty-two days for metepa-treated males, as compared with forty-nine days for untreated males. With gamma radiation, 6,500 rads or more reduced reproductive potential of males by 93%. Tests comparing reproductive potential of equal numbers of irradiated and untreated males competing for untreated females showed a 68–86% reduction in reproductive potential when theoretically only a 50% reduction was expected. Irradiated males up to three weeks old survived as well as untreated males; subsequently, irradiated males tended to die off at a faster rate than those in the controls. Dosages of 1,000 rads and higher completely sterilised irradiated females and above 1,000 rads killed all pupae less than eight days old at the time of irradiation. Although dissection showed that most of the dead puparia contained fully formed adults, there was no evidence that the dosages used increased either the incidence of malformed flies or mortality during the first day of adult life.

MASS-REARING STUDIES

Field and cage studies on rearing of *G. morsitans* within its natural habitat are being conducted at the Chirundu Field Station in the mopane woodlands of the Zambesi River valley. It had been suggested that since evidence indicated that continuous dense thicket provided a barrier to the movement of *G. morsitans* (Swynnerton, 1936), a roofless cage might prevent emigration of introduced tsetse flies. In contrast to the reports that led to this suggestion were those of flies resting at heights over 20 feet and of puparial deposits in holes high in trees. In testing whether the flies could be confined by a roofless cage a 17-foot wall was erected around an acre of tall mopane woodland by sewing cotton-gauze cloth to a wire framework supported by growing trees. Starting on 28 August an ox was tethered daily under a thatch shelter inside this cage and another under a similar shelter about 50 yards away outside the cage. Flies taken from these two animals were numerically coded with paints and released. From 8 September onward four oxen were tethered inside the cage from dawn to dusk. After 10 September catching and marking was carried out only for one day a week and the single outside bait ox was thenceforth present only on that day. On the evening of 5 September about 5,000 whole puparia were placed inside the cage on a sheltered tray. Puparia on this tray were covered with a layer of vermiculite mixed with Calco Blue dye and then with a layer of sand following the method of L. F. Steiner (personal communication). Emerging tsetse flies picked up the dye on the everted ptilinum and some dye was retained when the organ was withdrawn. These marked flies could be identified after recapture by squashing the head on filter paper and wetting with a drop of

acetone. By 8 October emergence had ceased and counts showed that 2,145 flies had emerged, the peak of emergence occurring between 15 and 30 September.

Of 1,988 *G. morsitans* collected within about a half-mile radius outside the cage up to 26 October, twenty-nine were marked with the dye. Occasional checks, during which a total of ninety-seven flies were caught, failed to recover any dye-marked flies inside the cage, although flies taken at the emergence tray were invariably well marked. Recapture of flies marked with paints also showed that the cage failed to prevent either egress or ingress of *G. morsitans*. The number of *Glossina* collected from bait oxen stationed inside and outside the cage is given in Table II. In spite of the evident failure of the cage to retain its population, the observations suggested that the installation of oxen at the site caused the build-up of an aggregation of *Glossina* independent of the effect of the introduced puparia.

Table I.—Number of *G. morsitans* progeny from 25 untreated females caged with 25 treated or untreated males (2 replicates).

Treatment	After pupal exposure		After adult exposure	
	Pupae	Adults	Pupae	Adults
Tepa	0	0	0·5	0
Metepa	0	0	0·5	0·5
Apholate	34·0	30·5	7·5	7·0
Control	21·0	19·0	24·0	23·0
6,000 rads	5·5	4·6	—	—
6,500 rads	1·3	1·0	—	—
7,500 rads	1·3	1·2	1·0*	1·0*
Control	18·7	16·1	—	—

* 1 replicate only

Table II.—Number of specimens of *Glossina* collected from bait oxen stationed inside and outside of roofless cage (1964).

Date	Time	Inside cage						Outside cage					
		G. morsitans			*G. pallidipes*			*G. morsitans*			*G. pallidipes*		
		♂	♀	T	♂	♀	T	♂	♀	T	♂	♀	T
Aug. 28	0900–1200	9	6	15	—	—	—	10	5	15	—	—	—
29	,,	22	9	31	—	—	—	28	6	34	—	—	—
31	,,	15	4	19	—	—	—	21	21	42	—	—	—
Sept. 1	0900–1600	11	8	19	—	—	—	30	30	60	—	—	—
2	,,	25	3	28	1	—	1	43	53	96	1	7	8
3	,,	16	3	19	—	1	1	37	17	54	6	5	11
4	,,	5	1	6	—	—	—	26	20	46	3	11	14
5*	0900–1400	12	6	18	—	—	—	36	25	61	1	4	5
7	0900–1600	15	12	27	—	—	—	37	33	70	3	12	15
8	0900–1800	28	6	34	—	—	—	45	18	63	20	19	39
10	0600–1830	55	51	106	1	6	7	40	23	63	37	14	51
17	,,	72	67	139	4	5	9	99	104	203	30	40	70
24	,,	120	123	243	12	18	30	112	66	178	32	52	84
Oct. 1	0530–1830	52	58	110	8	9	17	91	102	193	48	55	103
8	,,	20	35	55	—	3	3	76	73	149	29	17	46
15	,,	28	16	44	7	1	8	72	73	145	23	23	46
Totals		505	408	913	33	43	76	803	669	1472	233	259	492

* Pupae placed in cage after 14.00 hours

This conjecture was strengthened by the behaviour of *G. pallidipes* for which the mopane woodland was far from an ideal habitat, although suitable riverine thicket vegetation was within 150 yards of the cage. On the first four days of observations, no *G. pallidipes* were seen; but on 2 September, eight were taken from the outside and one from the inside ox. On 3 September, the numbers taken were eleven from the outside and one from the inside, but during the next four days none were taken inside. By 8 September the catch outside had risen to thirty-nine, and on 1 October, 103 and seventeen of *G. pallidipes* were taken outside and inside the cage, respectively. Although these preliminary data have not been fully analysed, there is an indication that small herds of cattle stationed at fixed points will serve as foci for aggregations of *Glossina*. Such aggregations are possibly composed of insects that visit the focus for a short time and then disperse once more in the manner suggested by Nash (1930). In addition, the failure of the 17-foot high wall to retain *G. morsitans* provides further evidence that these tsetse flies inhabit vegetation well above ground level.

DISCUSSION

Although much research effort remains, evidence is now available to show that practical methods can be devised to sterilise *G. morsitans*. Especially desirable are methods of increasing longevity of the sterile male tsetse fly, although in a field programme reduction of longevity could be overcome by adjusting the rate of release of sterile males. Ability to mass-rear this species, however, continues to be the greatest obstacle in utilising the sterile male technique; these investigations have not yet proceeded far enough to evaluate promise along these lines.

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AN EXPERIMENT ON THE ERADICATION OF *GLOSSINA SWYNNERTONI* AUST. BY INSECTICIDAL TREATMENT OF THE RESTING SITES

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INTRODUCTION

The principle of applying insecticide to vegetation to control tsetse flies has gained widespread acceptance. Thus the control of the riverine *G. palpalis*, described by Wilson (1953), is now a routine operation in East Africa. Much the same technique has been used in West Africa (Davies, 1958). *G. pallidipes* occupies a less restricted habitat but has been controlled by spraying Dieldrin emulsion along river banks and along paths cut through the forest (White, 1963; Thompson, Glover and Trump, 1960).

G. morsitans and *G. swynnertoni* occur over wide areas of savannah country covered by more or less dense bush. Aerial spraying would seem to be the most suitable technique (Burnett, Chadwick, Miller and Beesley, 1963) for eradicating fly in dense, continuous bush through which it is difficult to move. In thinner bush, aerial spraying is too costly a technique and residual spraying may be cheaper. Thus Blasdale (1960) and Hocking (1961) were able to attack *G. morsitans* with considerable success by applying insecticide to the resting sites.

G. swynnertoni occupies large areas of dry, open thornbush in Masailand which could be used for low density grazing if the fly could be eradicated at a sufficiently low cost. The resting sites of the flies appear to be relatively restricted (Chadwick, 1963) so that a selective spraying operation should be feasible. In the work described here this suggestion has been tested. To keep the cost down, simple methods and light inexpensive equipment has been used as far as possible. A subsidiary experiment, necessary to clear the area completely of flies, has involved the control of *G. pallidipes* which was restricted to a river bed running through the area. The technique employed in the control of this fly was that described by White (1963).

THE SITE OF THE EXPERIMENT

The area in which the experiment was carried out consists of a block of bush some 14 miles long and 35 square miles in area lying at the north-east corner of Lake Manyara in Northern Tanganyika (see map). The eastern end of the block consists of low parallel ridges on which the soil is thin and stony with a concrete-like conglomerate beneath. On the ridges the dominant tree is *Commiphora schimperi* with smaller numbers of *Balanites aegyptica*, *Acacia tortilis*, *A. mellifera* and *C. subsessifolia*. Between the ridges lie areas of " mbuga " grassland. In the

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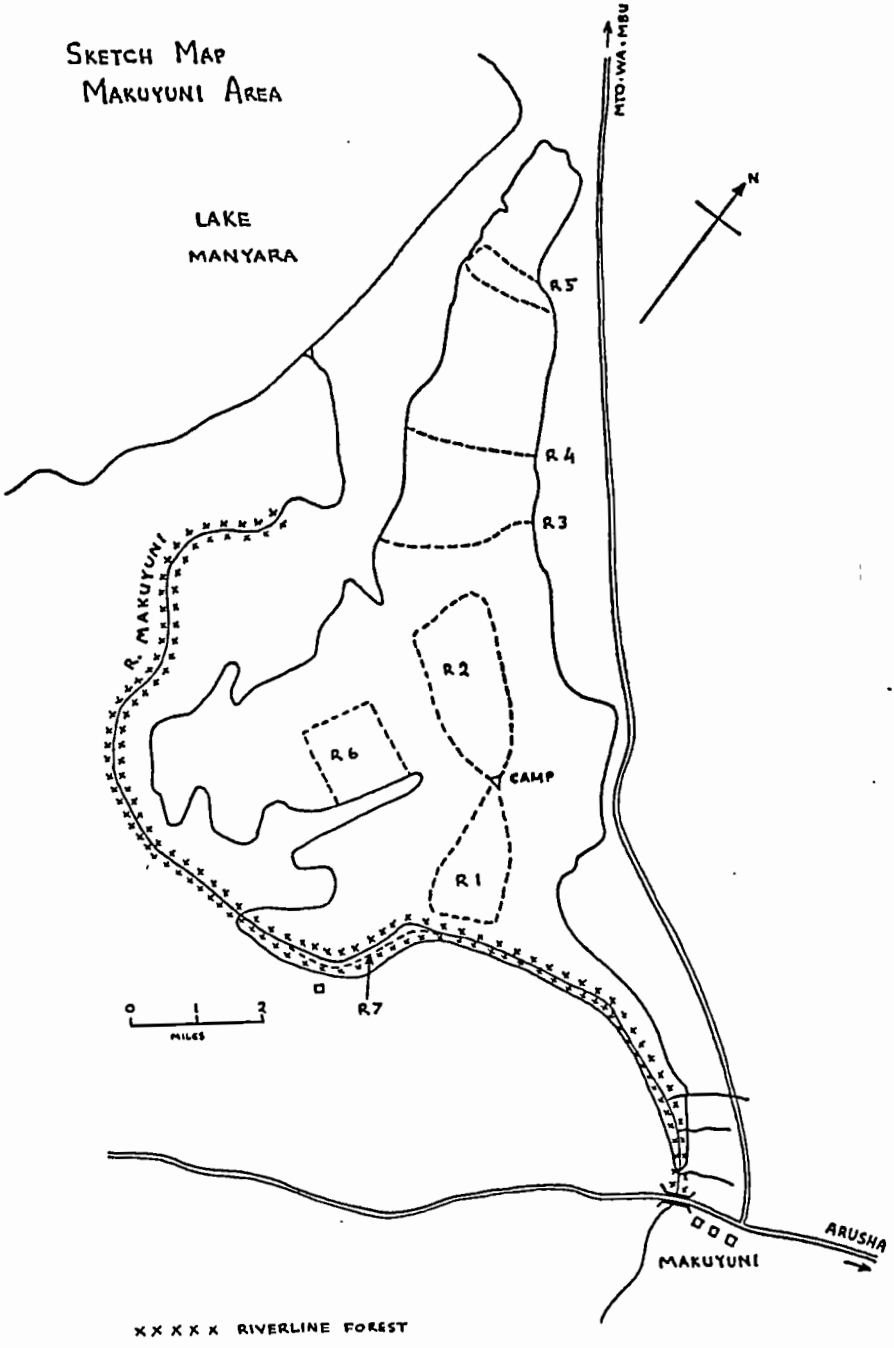
SKETCH MAP
MAKUYUNI AREA
LAKE
MANYARA
MTO. WA. MBU
N
R5
R4
R3
R2
R6
R1
CAMP
R7
R. MAKUYUNI
0 1 2
MILES
ARUSHA
MAKUYUNI
XXXXX RIVERLINE FOREST

centre of the block the bush is more even and continuous. At the western end, the ridge structure is less marked and the bush consists of glades with denser bush between and much of this has developed into thickets. In this area there are more of the last four species of trees. The ground surface is scattered with calcareous nodules derived from a period when the level of the lake was higher. The present western limit of the bush is formed by a bank of larger nodules up to a foot in diameter and impassable even to Land-Rovers.

The Makuyuni River, which only flows during the rains, runs along the south-east of the block and then through grassy plains. It is bordered by large fig trees with some *Hyphaenae coriaceae* palms farther away from the bank. *G. pallidipes* occurs throughout the tree-bordered portion of the river. Over part of its length, the river meanders considerably forming loops within which occur glades of *A. tortilis*, *B. aegyptica* and *C. schimperi*. *G. swynnertoni* was found in some of these glades. Approximately 20 miles length of the river were sprayed.

The block is moderately well isolated by grassland on all sides, but flies might enter from the Manyara National Park 1½ miles to the west or from an infested area to the south of the river and connected to the experimental area by scattered trees. The river was partially isolated by a cleared area to the east of Makuyuni village.

The game population is pleasingly large and varied. The animals seen included: lion, leopard, hyaena, jackal, serval cat, zebra, rhinoceros, warthog, giraffe, bushbuck, lesser kudu, eland, buffalo, duiker, waterbuck, reedbuck, kongoni, wildebeeste, dik-dik, impala, Grant's and Thompson's gazelles, oryx, steinbuck, monkey, baboon, monitor lizard and ostrich. Fresh elephant dung was once found but elephants were not resident. There was no evidence that the game was in any way affected by the spraying and no dead animals were found.

MATERIALS AND METHODS

Two insecticides were used. The extreme western portion of the block was treated with a 3% emulsion in water of thiodan (6,7,8,9,10,10-hexachloro-1,5,5a, 6,9,9a-hexahydro-6,9-methano-2,4,3,benzodioxathiepine-3-oxide) made from a 35% concentrate. The rest of the block, together with the river vegetation, was treated with a 3% emulsion in water of Dieldrin made from Dieldrex 15T concentrate.

The application of insecticide was rigidly confined to the known resting sites of *G. swynnertoni* in the area. These were almost entirely the undersides of the branches of trees. Study had shown (Chadwick, 1963) that flies were concentrated beneath branches at heights of between 4 and 9 feet, beneath branches of 1 inch to 4 inches in diameter and beneath branches sloping at less than 35 degrees. Insecticide was, therefore, applied only to sites falling within these limits regardless of the species of tree. This restriction ruled out a large proportion of the branches present and often it was only necessary to treat a foot or so of branch on a tree. Sufficient liquid was applied to reach a point just short of " run-off ".

The spraying team consisted of a Field Officer in charge, a driver with a long-wheel-base Land-Rover and seven spraymen. Kestrel knapsack sprayers were used to apply the insecticide. These machines have a 3-gallon polythene bottle mounted on a light tubular frame, a flexing diaphragm pump and an air bottle to give an even flow between strokes of the pump. The lance was fitted with a 45-degree bend, to facilitate spraying beneath branches and a size 00 Bray fan pattern

ceramic jet. The total weight of the sprayer is only 11 lb. when empty. A cloth sleeve was fitted over the lance handle to protect the right hand from falling droplets. The spraymen wore overalls and plastic hats. These, together with the lance sleeves, were washed regularly.

Whilst spraying, the party operated by moving across the width of the block with the sprayers spaced out in line abreast, coming closer when the trees were more dense. On the left or the right of the line the Field Officer followed a compass course and marked his passage with splashes of white paint on the trees. On the return course, the end man of the line followed the line of paint and was thus able to avoid leaving unsprayed gaps. The principal weakness of this procedure was found in very thick bush where it was difficult to keep contact down the line which had, therefore, to be shortened so that very little ground area was covered at a single passage.

The spraymen were this Institute's tsetse patrol staff who have had considerable experience in a variety of control work. They were introduced to this particular operation by making their own direct observations of the resting flies. This was followed by demonstrations and a day of spraying around the camp under individual supervision. For the first three days of operational spraying, three officers were present after which one of us (H.T.M.) was permanently in the block with weekly visits from one or other of the remaining authors. A particularly gratifying point was that one could often see the tsetse flying off the branches as they were sprayed. Spraying was stopped every few days so that the same staff could go over the fly rounds.

The river was treated with 3% Dieldrin using Motoblo mistblowers set to have a discharge rate of 1,800 ml./min. when the lance was held horizontal. The spray was aimed at vegetation up to a height of 8 feet on each side whilst walking along the river bed. In some parts of the river the vegetation on the banks was more extensive and the outer edge was sprayed as well to give a better chance of flies contacting a lethal deposit. Glades containing *G. swynnertoni* were sprayed in the same way as the main body of the area.

RESULTS

Entomology

The numbers of *G. swynnertoni* were followed by means of patrols on Rounds 1–6 (see Map), each of which was made up of 100-yard sectors. The mean numbers of flies caught each month per 10,000 yards of round are shown in Table I. Before spraying, the greatest numbers of flies were found in the centre of the block on Rounds 3 and 4 where the bush growth was most even. At the western end, which consisted of thickets with glades between them there were fewer flies (Buxton, 1955, p. 289) as was the case at the sparsely bushed eastern end where Round 1 was set out. Round 6 was opened to sample the population in an almost pure stand of *Commiphora schimperi* where flies were present in large numbers. The proportion of young flies in the catch on this round was unusually high with a mean of 27% for the three months July to September compared with 9% for the adjacent Round 2 where the bush was more varied.

The numbers of *G. pallidipes* were sampled using a cattle round, No. 7, running along the bed of the river and having fifty sectors of 100 yards. The numbers of flies caught and the composition of the catch varied considerably on

Table I.—Mean catch of flies per 10,000 yards
Rounds 1-6, *G. swynnertoni*
Round 7 *G. pallidipes* caught using a bait ox

	Pre- or post-spraying	Rounds						
		1	2	3	4	5	6	7
February	pre	16·3	135	289	—	—	—	—
March	,,	28·5	139	390	422	—	—	—
April	,,	28·0	174	362	274	—	—	—
May	,,	20·0	167	300	254	82	—	—
June	,,	15·7	144	222	272	82	—	—
July	,,	17·1	158	271	252	81	232	84
August	,,	19·2	115	287	223	50	216	150
	post	—	—	—	—	6·5	—	—
September	pre	10·5	120	100	141	—	86	105
	post	—	1·1	1·2	0	1·7	—	—
October	pre	5·8	—	—	—	—	—	—
	post	0	1·5	0·8	0	0	1·7	2·0
November	,,	0	0·3	0	0	0	0·4	1·0
December	,,	0	0·3	0	0	0	0	0
January	,,	0	0	0	0	0	0	0·4

Table II.—Catches of *G. swynnertoni* on Round 2 before and after spraying

Date	Old males	Young males	Old females	Young females	Total catch	Apparent density
21 August	78	4	6	7	95	89
28 August	84	11	9	4	108	96
6 September	85	8	9	5	107	97
16 September	90	7	4	3	104	102
	Area sprayed 23–26 September 1963					
28 September	0	0	1	0	1	0
7 October	1	2	0	1	4	1·1
21 October	0	0	0	0	0	0
28 October	0	0	0	0	0	0
4 November	0	1	0	0	1	0
9 November	0	0	0	0	0	0

this round. After the rains in November the river bed was too wet to use and a new round had to be set out on the east bank of the river alongside the old one. The catches from December onwards are, therefore, not strictly comparable. After spraying, some additional surveys were made in the more distant parts of the river, without using the bait ox, but no flies were found.

Spraying started at the western end of the block where the thiodan was used. Unfortunately, one of the thiodan drums had been damaged during shipping and had leaked with the result that only half the area covered by Round 5 was treated with thiodan before the supply ran out. The early disappearance of fly from this round shows, however, that thiodan was effective. Additional surveys made at random through the area treated with thiodan also failed to find any flies. Dieldrin

was used to treat all the rest of the block and also was effective. The mean catches on the various rounds for each month are given in Table I. During the spraying period these have been divided into pre- and post-spraying figures. The catches can be put into three groups. Before spraying the figures show the expected seasonal variation and only the catches of about 400 flies per 10,000 yards on Rounds 3 and 4 in March 1963 are noteworthy in view of the scattered nature of the bush. After spraying, there is a period of five weeks during which non-teneral flies may be caught during the few days immediately after spraying and then only teneral flies are found. This period is shown in more detail in Table II which gives the catches on Round 2. On Round 4 no flies at all were caught after spraying. On several occasions, it was noticed that one could walk up behind the advancing line of sprayers and be free from flies, overtake the sprayers and immediately be attacked as one entered the unsprayed area. This suggests that the majority of the flies were being immobilised within a few minutes even of applying the spray. The third group of catches covers the period after all the flies, which escaped the spray as pupae, have already emerged so that no flies are caught. For reasons given in the discussion, the single fly caught on Round 2 in December is regarded as being derived from an immigrant parent.

Comparison of the results on the individual rounds suggests that when the sprayed area is close to an unsprayed round, the catches on the round fall somewhat. This is what might be expected when the normal to-and-fro movement of flies is changed into the total loss of those emigrating from the unsprayed area. Further detailed observations on this point would be of interest since they would provide support for the treatment of strips of resting sites only instead of treating the whole area (see Discussion).

No rain fell during the spraying period, the first slight shower occurring on 6 November. The grass over most of the block was burned on the night of 1 September when a fire, started at a charcoal burner's camp at the extreme east end of the block, ran very quickly down to the west driven by a strong wind. Bioassays of bark taken from a tree before and after the fire showed that the deposit of Dieldrin had lost little of its toxic effect. The number of flies in the unsprayed areas was considerably reduced by the fire as the pre-spraying catches for September in Table I show.

The river, together with some of the adjacent bush at the eastern corner of the block, was sprayed during the second half of October and only teneral flies were found after the middle of the month. During the second half of November the unusually heavy short rains flooded the river, which was as much as 15 feet deep in places, so that it was not possible to do the cattle round. The rain and flooding probably removed the greater part of the Dieldrin deposits, so that some pupae deposited before spraying may have produced flies which were not exposed to a lethal deposit on emergence. The numbers of flies caught on the original round in the river bed during July, August and September were rather irregular, but averaged 113 flies of all stages per 10,000 yards for the three months. One fly was caught in January on the new round so that the reduction in catches for December and January combined was 99·904% using the July–September period as a base-line.

SPRAYING OPERATIONS

The principle of using simple techniques and equipment to obtain rapid coverage of the block seems to have been successful. The work started slowly,

mainly because of the very thicketed nature of the bush. All the potential resting sites within the limits described and which could be reached were treated. This meant slowly forcing a way into the thorny thickets, spraying the few sites within reach and then moving on a few feet. Furthermore, the Land-Rover carrying the insecticide and the water drum had to stay outside the bush so that time was wasted in walking back to refill the sprayers. In future work a technique of spraying only the outside edges of the thickets might be tried. Once away from the thicketed area, the work went very much faster and areas ranging from a quarter to one square mile a day were covered, according to the density of the bush. The Land-Rover gave close support and little time was wasted on refilling. Using the Land-Rover in this way resulted in several tyres being damaged by stumps and sharp stones cutting through the cords, so that the tyre had to be scrapped before the tread was worn. This problem might be overcome by using tyres of the steel reinforced stabilised tread type, but these were not available.

The Kestrel sprayers gave little trouble relative to the hard use they received. Whilst working in the thickets, some pipes were punctured by thorns. This was cured by wrapping the pipes in polyvinyl chloride insulating tape. The rubber washers in the lance valves sometimes expanded and jammed and so were replaced. The pump diaphragms had to be replaced regularly, since the rubber became denatured and broke away from the canvas backing. An improved type of diaphragm would be desirable. The cost of replacement parts is included in Table III. Weighing only 11 lb. these sprayers were very much less tiring to use than other types and furthermore, being simple, gave freedom from the difficulties of starting small two-stroke engines or recharging pressure-retaining sprayers. At £17 5s. 0d. each, they were also cheap to buy.

Table III.—Costs

	£
RIVER SPRAYING 20 MILES	
Dieldrex 15T, 60 gallons	114
Spraying team wages	33
Fly round costs	10
Machine depreciation	20
Transport	40
Petrol and oil for Motoblo's	4
Field Officer	30
Total Cost	£251
Cost per mile	£12·5
BUSH SPRAYING 35 SQUARE MILES	
Dieldrex 15T, 375 gallons at Shs. 38/- per gallon	713
Thiodan 35% E.C. at Shs.75/- per gallon (15 gallons)	60
Spraying team wages	155
Fly round costs	55
Transport	·236
Six tyres at £10	60
Quarter cost of 7 Kestrel sprayers (assuming four seasons' use)	30
Sprayer spares	17
Field Officer	170
Total Cost	£1,496
Cost per square mile	£42

A strong wind blew during most of the spraying period and it was necessary to replace the size ooo jets, used initially, by the larger size oo which gave a coarser spray which was deflected less by the wind. Since this wind continued to blow at the usually calm periods of dawn and before dusk, aerial spraying would have been impossible. After the grass had been burned in the fire on the night of 1 September, it was very much easier to move about the block. In future work, deliberate burning of the grass as soon as it is dry might be worth while.

COSTS

Approximate costs for the operation are given in Table III. The cost of surveys, camp building, supervision from Arusha and fly rounds before and after the spraying period have not been included. Expenses for spraying the river against *G. pallidipes* and the bush against *G. swynnertoni* are given separately, although it has been necessary to make an arbitrary allocation of costs for fly rounds, depreciation and transport for the river spraying.

DISCUSSION

The population of *G. swynnertoni* in the block appears to have been eradicated, so that the experiment can be regarded as a success. A rather longer period of observation is desirable before a final assessment is made and fly rounds are being continued. The very rapid disappearance of fly from each round as it was treated provides strong support of the observation that *G. swynnertoni* rest beneath branches within a limited range of heights, diameters and angles of slope during the day. In theory, flies of the original population might be found for eighteen days after spraying, whilst pupae deposited by the last of these flies could, after a period of, say, thirty days as the pupal life, produce flies which might survive for a further eighteen days (Chadwick, 1963). Thus, non-teneral flies might be found for as long as sixty-six days after spraying. In practice, this does not seem to occur and extinction of the original population is very much more rapid as is shown in Tables I and II. Subsequently only newly emerged flies are caught. The single fly caught on Round 2 on 23 December is worth noting. It was found eighty-eight days after the area was sprayed and was a teneral female. The pupa must, therefore, have been deposited about 20 November, nearly two months after the area was sprayed. The parent fly is thus most unlikely to have been derived from the original population, which indicates immigration. It is known, furthermore, that a hunting party travelled between the infested country to the south of the river and this part of the block at about this date so that they could have carried the parent fly with them. The increased amount of travelling to and fro amongst the Masai in January makes further immigration likely, although this may be slowed by the spraying of the scattered trees to the south.

The costs of this operation compare favourably with previous ones. Hocking (1961) found a cost of £250 and a Dieldrex consumption of 76 gallons per square mile in controlling *G. morsitans* in Uganda. Kirkby and Blasdale (1960) used DDT to control fly in Nigeria at a cost of £98 per square mile, and this was subsequently reduced to £86 (Davies and Blasdale, 1960) by using a lower DDT concentration and being more selective in spraying the resting sites. In the present experiment against *G. swynnertoni* the cost was £42 and the insecticide consumption, assuming that Dieldrex was applied to the whole block, was 11·6 gallons per square mile.

The even lower cost of £26 per square mile has been recorded by Robertson (1963) in the control of *G. morsitans* in Southern Rhodesia. In this particular instance, it was possible to exploit the effects of the severe hot, dry season during which the fly is confined to the narrow strips of bush along drainage lines and to spray only the boles of the trees, and the undersides of the branches up to 12 feet, along these drainage lines using motor knapsack sprayers. The actual area sprayed was, therefore, very much less than the area rendered free of fly so that the costings are not really comparable.

Several further economies in the control of *G. swynnertoni* would seem to be possible, although their extent could only be found after more experience. A lower Dieldrin concentration of 2% might be used. Fewer branches might be sprayed through narrowing the limits used. For example, the height limits could be placed at 5 to 8 feet instead of the 4 to 9 feet used in this experiment. This might, of course, lead to a slower extinction of the population since the probability that a fly would land on a treated surface would be smaller. Where thickets occur, only the outer edges might be treated. This would ease the work by a considerable amount, since forcing a path through a thicket, to spray inside it, is a slow process. Finally, in open bush, it may be possible to increase the spacing between spraymen so that a series of strips, in which the resting sites are treated, is laid down with untreated strips in between. All these possibilities, with the exception of the first, amount to cutting down the proportion of the resting sites which are treated with insecticide. Perhaps it will be found that this proportion has to be varied with the nature of the bush and the fly density.

The spraying of the river vegetation against *G. pallidipes* seems to have been moderately successful. A single fly was found in January. This also may have been an immigrant since the isolation of the eastern end of the river is certainly not absolute. On the other hand, the flooding and the heavy rain soon after spraying make it possible that some of the original population of fly and pupae could have survived. In this case also, longer observation seems necessary. As it is, comparing the July–September mean catch with the December–January catch indicates a 99·904% reduction.

Acknowledgements

We are indebted to Messrs. Farbwerke Hoechst of Germany for the gift of the thiodan used and to Mr. George Damm, of Manyara Ranch, for permission to work on his land on which the eastern end of the block lay. Mr. D. B. Turner, Regional Development Officer, originally suggested the area as a site for experiment and took a great interest in the operation.

POSTSCRIPT

24 February 1964

Further fly rounds on the river show that *G. pallidipes* is reinfesting the river strip. Thus on Round 7, one fly was caught on 19 February and two on 21 February. A further survey, using a calf small enough to be carried in the Land-Rover, made to the east of Round 7 showed larger numbers of flies. It would seem, therefore, that the reinfestation is due to flies moving along the river from Makuyuni and that catches on the normal Round 7 can be expected to rise to a level approaching the

pre-spraying figure within the next few months. This emphasises the importance of adequate isolation in insecticidal control of tsetse.

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AERIAL APPLICATIONS OF INSECTICIDES IN EAST AFRICA

XIV

VERY LOW VOLUME AEROSOL APPLICATIONS OF DIELDRIN AND TELODRIN FOR THE CONTROL OF *GLOSSINA MORSITANS*

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Arusha, Tanganyika

INTRODUCTION

Two earlier experiments on the aerial control of tsetse fly at Chungai, in the Central Region of Tanganyika, have been described in this series of papers (Foster, White and Yeo, 1961; Burnett, Yeo, Miller and White, 1961). These authors concluded that further progress would depend on using more efficient dispensing equipment giving a reduction in volume dosage, or on using a more potent insecticide. By 1962, both had become available. New equipment was provided by a simple exhaust aerosol-generator fitted to the aircraft owned by this Institute and the new insecticide Telodrin was on the market at a price competitive with Dieldrin. At Chungai, the South Block remained untouched with a moderately high population of *Glossina morsitans* Westw., and the North Block, from which the fly had been almost eliminated in 1960, had been repopulated from the South Block across the old barrier clearing, now much overgrown. Experiments were planned to test the new equipment using the familiar Dieldrin on one block and to compare Telodrin with Dieldrin on the other, also using the new equipment.

This experiment was carried out at the suggestion of and in co-operation with the Department of Tsetse Control, Tanganyika Government, who provided most of the insecticide and helped in many ways.

METHODS

In the successful 1959–60 experiment, a 20% concentrate of Dieldrin in involatile oil was diluted with seven parts of power kerosene to give a 2½% solution dispensed at 0·125 gal./acre (1·4 l/ha) (Burnett *et al.*, 1961). The primary intention of diluting was to maintain a fairly high volume dosage because it was thought that one reason for the lack of success the previous year was the low volume dosage of 0·08 gal./acre (0·9 l/ha) (Foster *et al.*, 1961). It is now known that a more likely reason is the low inherent toxicity of B.H.C. to *G. morsitans* (Burnett, 1961 a) and consideration of the known rapid evaporation of power kerosene from aerosols (Yeo and Thompson, 1954) suggested that the principal function of the kerosene was to produce droplets much smaller than the rotary atomisers could produce directly; evaporation of seven-eighths of the droplet would reduce the diameter of every drop produced to one half. Successful though this method was, it was wasteful. Although the diluent was cheap, it was expensive to transport in large amounts and the small aircraft could carry enough diluted spray to cover only a small area

and had to land to reload while spraying conditions were at their best. If the required small droplets could be produced from the concentrate, the diluent could be omitted with savings in direct cost, in transport charges and in non-productive flying. It was decided to use only the minimum amount of diluent required for each insecticide formulation to give the desired drop spectrum with the new equipment, while keeping the area dosage of Dieldrin the same as in 1959–60 and fixing that of Telodrin in inverse proportion to its relative toxicity (about 2·5; Burnett, 1961 a). The performance of the equipment was to be assessed before the experiment started and the operational procedures used in 1959–60 were retained. It was anticipated that the replacement of the rotary atomisers by the more compact exhaust generator would improve the handling of the aircraft and permit higher speeds, thus increasing daily coverage.

The aircraft used was a Cessna 182E high wing monoplane fitted with a 90-gallon Sorensen belly-tank, a windmill-driven pump and an 18-inch extension to the exhaust pipe. The spray liquid was fed into the exhaust extension through a metering jet and produced a fine aerosol. The aircraft was flown at a true air speed of 130 m.p.h. and covered successive swathes on northerly and southerly courses at intervals of 52·5 yards. Each day the first run was made over the same marker as the last run of the previous day in order to compensate for errors in tracking and changes in wind direction that might lead to gaps in the coverage.

The nominal dosage of Dieldrin was the same as in 1959–60, 0·5 oz. (14 gms) per acre, and it was originally planned to use the same 20% solution without further dilution at $\frac{1}{64}$ gal. per acre. However, the Tanganyika Government was prepared to supply Dieldrex 15T, an 18·4% emulsifiable concentrate, free of charge from stocks in hand. Although the characteristics of this formulation when disseminated through our equipment were unpredictable, it was decided to use it after testing, possibly with the addition of other oils. Telodrin was available only as a 10% solution in an involatile oil formulated and supplied by the Shell Chemical Co. of East Africa Limited.

Assessments of these formulations when emitted by the aerosol equipment were interrupted by the wrecking of the aircraft while on other work. By the time a replacement was in service the experiment was already a month overdue and in danger of carrying on into the rains at the end of the year and there was no time for proper assessment. It was decided to make an application and assess the results empirically from catches of tsetse before and after spraying, testing modifications in procedure in the same way. The first application of the Dieldrex 15T gave a poor kill. This was attributed to excessive evaporation of the solvent, and Shell " diesoline " was added to increase the droplet size after evaporation. The addition was limited by maximum emission rate of the dispensing equipment to one part of diesoline to two parts of Dieldrex 15T. The mixture, containing 12·3% Dieldrin, appeared to be effective when applied in the field and was used for all but the first application at 0·0254 gal. per acre. Telodrin was used throughout as the 10% solution at 0·0124 gal. per acre.

The South Block held more tsetse than the North and was considered more useful, and was therefore used to test the new equipment, dispensing the proven Dieldrin. The North Block was treated with the untried Telodrin. The application to the two blocks followed each other without a gap and the intertreatment interval on each block was fixed at three weeks. The reduction from the four weeks used in 1958 and 1959 was made for the reasons given by Burnett (1961 b).

The aircraft flew over mobile markers, one of which marked along each edge of the block, and flew on compass bearings. Various types of beacons were tried

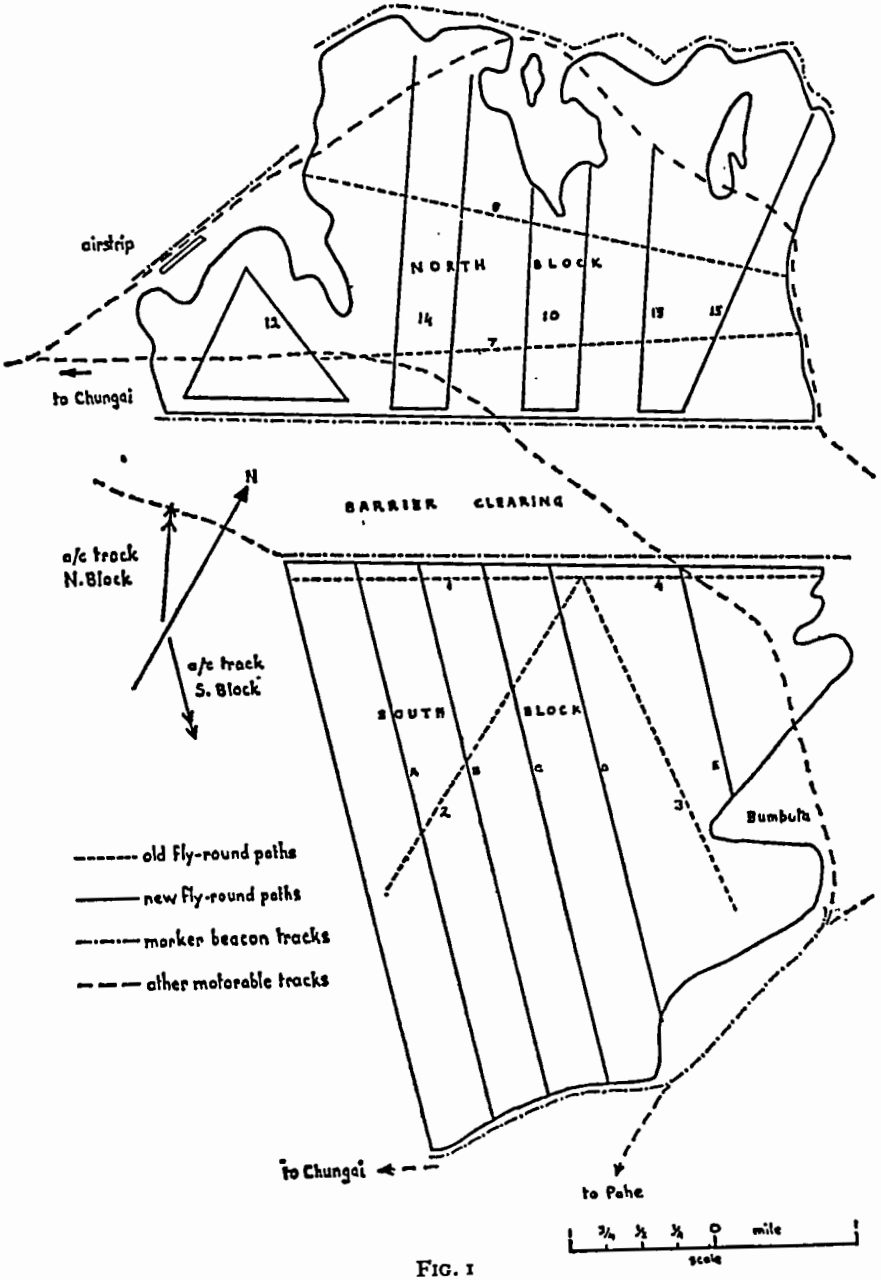

FIG. 1

out and are discussed elsewhere (Miller and Chadwick, 1963). A successful attempt was made to apply insecticide after dark, at full moon.

DESCRIPTION OF THE AREA

A sketch map of the area is given in Fig. 1. The vegetation has been described by Foster, Yeo and White (1961), from a survey carried out by D. B. Turner. Both blocks are about 11 square miles in area and consist for the most part of *Acacia commiphora* thicket with glades and, in the North Block, extensive areas of *Acacia tortilis spirocarpa* woodland. Before spraying started, some reclearing of growth in the mile-wide barrier was done by the Tanganyika Government, but this was not extensive and it was not possible to burn the barrier until the time of the fourth spraying when grass fires were widespread. Since 1960 some further settlement had taken place in the barrier clearing and the western side of the North Block in the region of Round 12 had been largely cultivated since 1959. Scattered but small areas were being cleared throughout the period of the operation in the South Block and continuous areas had been cleared along the road running through the block to Bumbuta. Game was not plentiful in the area, although rhinoceros were often seen on the rounds in the South Block.

The area was well isolated from other tsetse-infested areas. The nearest infested bush, Mauno (the Control Area), lay 3 miles from the south-east corner of the South Block across " mbuga " grassland and cultivated ground on Solare ridge. There was also a possibility that the Masai, driving cattle from Chubi further to the north-west, might bring a few flies to the North Block. This experiment had the advantage over the two earlier ones that the simultaneous treatment of both blocks, even if only moderately successful, would prevent significant migration into the treated areas.

ENTOMOLOGICAL ASSESSMENT

Numbers of tsetse were assessed by the usual method of standard fly rounds used in all earlier experiments. Special rounds for *G. pallidipes*, using cattle or screens, were not carried out and all catches of this species were made on the usual *G. morsitans* rounds. The staff at the Tanganyika Tsetse Department had made regular catches on the old paths 1–4 in the South Block and No. 7 in the North Block (see map) for many months but these were not suitable for aircraft purposes. Five new paths of a total length of 28,000 yards were cut in the South Block parallel to the proposed spraying track of the aircraft. This is important for accurate measurement of kill per application. In the North Block some of the fly rounds used in the last experiment were reopened and renumbered. They are shown in Fig. 1 and totalled 33,000 yards in length. Patrolling of the new rounds started in June 1962. Round 7 in the North Block was continued to provide continuity with the earlier observations, but the pattern in the South Block was too different for this to be done. In July two sets of rounds a week were planned. In the period covered by the insecticide applications two rounds were planned in each week following each application. For the first three applications in the South Block this was supplemented by daily rounds for three to four days on each path as it was covered by aerosol in an attempt to measure the mortality caused by each application. This procedure was given up when numbers fell too low for it to be worth while. Two rounds per week were done in both blocks until the end of January 1963, after which the frequency was reduced. For control figures the Tsetse Department supplied catches made at Mauno, 3 miles to the south-east. We were not aware until too late that this area had been influenced by selective clearing, but the catches are, despite this, of value.

An attempt was made to measure pre-treatment numbers of fly in both blocks by the marking and releasing method (Jackson, 1948). This suggested a population of about 5,000 flies in the South Block. Recaptures in the North Block were too few to make any estimate.

RESULTS

Operational observations

The experiment started later than planned as a result of the crash of the original aircraft, the first application starting on 30 July (see Table II). Detailed investigation of the North Block had shown that there were too few tsetse present for a good experiment, but it was treated not only because we were committed to the Tanganyika Government to treat the block but also to prevent return migration to the South Block. There were, in any case, sufficient fly to prove gross inadequacy in the operation.

Applications were characterised by high winds and poor visibility for the first few treatments. On the North Block the planned second application was missed due to fatigue-cracking of a weld on the exhaust-manifold of the aircraft, attributed to the experimental design of the dispenser; the planned fourth application was abandoned because of high winds when only about 60% of the South Block had been covered and the North Block not started. The interval to the next application was reduced to two weeks and both blocks successfully treated. The planned seventh treatment (that is, the actual fifth on the North Block) had to be postponed for three weeks because the airstrip was flooded. The planned eighth was made three weeks later. Thus, the South Block was treated eight times although the fourth was incomplete (but covered the most important part). The North Block received six full treatments, at somewhat irregular intervals.

The use of a concentrated spray greatly eased the operating problems, because there was less material to transport to the airstrip and because the aircraft was able to carry enough material for a sortie lasting as long as weather conditions were ever suitable. Thus no time suitable for spraying was lost in refilling the spray tank. Spraying was stopped if the mean wind speed rose to 12 feet per second and remained steady, but if the wind was gusty and therefore turbulent, spraying was stopped at lower mean speeds. As found in earlier operations, the aircraft was itself the most sensitive indicator of turbulence. Some guidance could be obtained from watching the aerosol cloud and the smoke from fires.

Flying

These were the first tsetse experiments in which the Cessna 182E aircraft was used. Very little time was available for experimenting with the novel exhaust aerosol before starting the experiment, and the gear was therefore very hastily designed and put together, and it was perhaps fortunate that so little trouble was actually experienced with it in the field—a testimony chiefly to its simplicity.

The exhaust aerosol generator has one great advantage over other forms of spray equipment in that the aircraft is relatively " clean ", and behaves in a nearly normal manner. It was possible to fly at a true airspeed of 130 m.p.h., the engine being operated well below its normal maximum cruising power to achieve this.

The plan of operation called for parallel tracks to be flown 52·5 yards apart. To assist the pilot in this, the normal Cessna compass was removed, and a British

P.12 Inverted Compass installed in the roof of the cockpit. The marking parties used Aldis Lamps as well as boards, and owing to the nature of the terrain the pilot was often able to see both lamps when lining up, which made accurate tracking very simple. Flying on the P.12 compass also produced accurate tracking, small errors tending to cancel out more often than not. Occasionally these errors would add up, and then a relatively large error in the tracking would occur. It must also be remembered that the marker lines were not laid out by trained surveyors, and there were bound to be some errors " built-in " to the layout.

In the early part of the experiment, when the weather was poor with bad early morning visibility, it was very difficult for the pilot when turning between runs, and picking up the markers would have been virtually impossible without the use of Aldis Lamps.

It was possible to carry enough insecticide to allow for nearly three hours' spraying in one sortie. The pilot found, however, that about 2½ hours per sortie was enough. The " g " forces in the turns, coupled with the concentration required to steer accurately, produced fatigue after 2½ hours, which resulted in less accurate flying.

An opportunity was taken to try night spraying when conditions were suitable at the full moon. No particular difficulty was experienced in this. Because of deep atmospheric inversions it was possible to fly considerably higher than in daylight without wasting insecticide, making flying relatively easy. Ideally, specially fitted searchlights would assist the pilot in avoiding obstacles like the occasional tall tree—the normal aircraft landing lamps are not much use for this, as they are set at the wrong angles. Night operations, of course, require the use of lights as swathe markers.

Entomological observations

Apparent densities (numbers of non-teneral males caught per 10,000 yards of fly-round path) are given in Table I for *G. morsitans* on rounds 1–4 in the South Block and on round 7 in the North Block for about two years before the experiment started. The A.D.'s for Mauno are also given from January 1961. In Tables II and III are given mean catches and A.D.'s per round for each block from two months before to twelve months after the insecticide applications. There was a slow fall in catches in the South Block during 1960–1 and a steeper fall in early 1962 that can be attributed to the very heavy and persistent rains of this season. In the North Block there was a very rapid increase in A.D.'s in 1961 probably due largely to the known immigration from the South Block, but detection of further increase was confused by superimposed seasonal changes; also immigration probably became less important as the A.D. in the South Block fell. These catches in Table I cannot be equated directly with those given in Tables II and III, but in the case of the South Block (Table II) the apparent fall from an A.D. of 37 in May to 26 in June is probably because a greater proportion of the new paths used in the latter month lay in lightly infested bush. The main centre of infestation in this block was about the northern area sampled by three of the old paths 1–4. In the North Block round 7 was covered on 30 July, just before the first insecticide application, and showed about the same A.D. as in May. As stated earlier, the control A.D.'s are of little use for indicating long-term natural trends because the area concerned had suffered selective clearing, but it is at least safe to say that between July 1962 and January 1963 there was no natural reduction in the population.

It was found that there were very considerable day-to-day differences in catches in the South Block in June and July 1962, and for the purposes of calculating the mortality inflicted by the first treatment, the mean catch of old males per round in July is taken (59·3). The post-treatment catch was high on the first day, fell for the next two, and then rose again. This rise is unlikely to be due to post-treatment emergence of new flies because the teneral percentage does not rise. It could be due to recoveries from a sub-lethal dose, but it is more likely that the differences are of the irregular nature found before the experiment started. Because delayed deaths due to Dieldrin are well known (Burnett *et al.*, 1961; Burnett, 1961 a) the first day's post-treatment catch has been omitted and the mortality calculated from the mean for days two to four (15·0) giving a kill of 75%. A similar calculation with females gives a kill of 67%. This was not satisfactory and the aerosol formulation was modified for the second application. For this, the mortality of males has been calculated from the mean pre-treatment catch for two weeks (14·75) and the mean post-treatment catch for days two and five (0·5). The result is 96%. This is an unsatisfactory figure for the exact mortality, being based on too few post-application captures, but if a χ^2 test is carried out to compare the mortalities obtained by the first two applications, based on the four fly rounds done before each treatment and those done two and three days after treatment, the result is significant at 2·5% (method of Fisher and Yates 1953, Table VIII). This is not conclusive because there are so many influences affecting tsetse catches, but it confirms the probability that the change in the formulation increased its effectiveness. This view is supported also by the final result of the experiment, because applications giving kills of the order of 75% of old males have never succeeded in reducing catches of *G. morsitans* to zero in earlier experiments.

In the North Block, once fly patrols were extended to the whole block, it was found that catches on round 7 had exaggerated the recovery in numbers (compare A.D.'s for July 1962 in Tables I and III). The female percentage is unusually high in this block. This is frequently taken to indicate a population under stress, but in such a case the teneral percentage is usually high as well, but this was not so in the North Block. No satisfactory measurement of kill in the North Block is possible, the numbers of fly being too small. The persistence of survivors of this small population until after the fourth application might suggest a kill inferior to that obtained in the South Block, but the long intervals of six and five weeks between the first three applications could be sufficient reason. The high female and teneral percentages immediately after spraying indicate heavy mortality of old males. In the South Block these percentages rose appreciably only after the second application, which supports the view that the first application there had little effect.

After the third application in each block few flies were caught and these were mostly tenerals. The last *G. morsitans* was caught in November 1962. No further flies of this species were seen until the middle of November twelve months later when two old male flies were caught, one in the North and the other in the South Block. They were found in the vicinity of a large herd of elephant that, with the start of the rain, had very recently moved from Mauno into the blocks, where they stayed for a few days and then moved north to Chubi. It is likely that these flies were brought by the elephants. No flies were found in December. The mean A.D.'s for the first year following the end of the insecticide applications work out at 0·0073 and 0·0062 for the South and North Blocks respectively.

In addition to the *G. pallidipes* shown caught in the South Block, a *G. swynnertoni*

was captured there in July and another in the first week of August 1962. In the North Block, two *G. pallidipes* were captured in June and a single one in August 1962, whilst three *G. swynnertoni* were found before spraying and a total of seven during the spraying period, the last on 18 December. Subsequently, an old female was caught on 18 August 1963. This scattered occurrence suggests continued immigration rather than residence and breeding. It is possible that these flies are brought by the cattle which are driven past the block on the way from Chubi waterhole, where fly occur, to Kolo market.

COSTS

Details of the costs of the two experiments are given in Table IV, together with those for 1959 for comparison. Calculations are made assuming that eight full applications were made in each case, using throughout the formulations and procedures used in the later part of each experiment. Basic costs are those that

Table I.—Apparent density of *G. morsitans* in South and North Blocks at Chungai and at Mauno control area. Catches were made on rounds 7 in the North Block and rounds 1–4 in the South Block. There are three rounds at Mauno for which the mean has been taken. All catches supplied by courtesy of Mr. F. McBain, Tsetse Field Officer, Central Region, Tanganyika, except for North Block in 1960

Month	South Block 1960	North Block 1960	South Block 1961	North Block 1961	Mauno 1961	South Block 1962	North Block 1962	Mauno 1962	Mauno 1963
Jan.	—	0·2	143	—	146	97	flooded	108	24
Feb.	—	—	106	—	130	85	,,	70	29
Mar.	—	—	92	4	93	76	19	45	19
April	109	0·2	55	12	86	56	14	27	11
May	148	0·3	116	17	87	37	14	20	13
June	158	0·4	94	19	93			7	Sprayed
July	180	—	82	28	48		11	12	by
Aug.	149	—	105	—	43	New rounds see Tables II & III		24	Dept.
Sept.	154	0·1	103	14	33			27	of
Oct.	130	1·4	132	13	93			32	Tsetse
Nov.	139	2·7	115	42	79			33	Control
Dec.	133	—	93	52	132			26	

Table II.—South Block: Mean catches of *G. morsitans* and *G. pallidipes* per set of fly rounds

Period 1962–3	No. sets of rounds	Old males	Old females	Tenerals m & f	Females %	Teneral %	A.D.	*G. pall.* all flies
June 1962	4	70					26	12
July 1–7	1	79	5	7	6·2	8	27	1
8–16	2	55·5	5	6	8·3	9	20	0
17–21	2	55	4	6·5	6·8	9·9	20	6
22–29	3	58	3	5	4·9	7·6	21	5

Period 1962–3	No. sets of rounds	Old males	Old females	Tenerals m & f	Females %	Teneral %	A.D.	*G. pall.* all flies
30 July–1 August FIRST insecticide application								
1 day post. app.	1	32	4	4	11·1	10	11	6
2 days post. app.	1	14	0	0			5	0
3 days post. app.	1	10	2	1	8·2	2·0	3·6	0
4 days post. app.	1	21	2	0			7·5	0
Aug. 8–13 .	2	22·5	2	2	6·8	4·8	9·2	3
Aug. 14–20 ..	1	7	1	1	12·5	11·1	2·5	1
21–23 August SECOND insecticide application								
1 day post. app.	1	3	1	0			1·1	0
2 days post. app.	1	1	1	0			0·4	0
3 days post. app.	1	0	0	0	33	52	0	1
Aug. 27–Sept. 9	2	1	1	2·5			0·4	0·5
Sept. 2–10 .	2	2	0	4			0·7	0·5
11–13 September THIRD insecticide application								
1 day post. app.	1	1	0	1			0·4	0
2 days post. app.	1	1	1	1			0·4	0
3 days post. app.	1	1	1	0	39	24	0·4	0
Sept. 18–23 .	2	1	0·5	1			0·4	0
Sept. 24–Oct. 1	3	1	0·67	1·7			0·4	0
2–5 October FOURTH (incomplete) application								
Oct. 3–9 .	2	0	0	1	100	80	0	0
Oct. 10–16 .	2	0	0·5	1			0	0
16–18 October FIFTH insecticide application								
Oct. 19–22 .	2	0·5	0	0·5			0·2	0
Oct. 23–29 .	2	0	0	0		67	0	0
Oct. 30–Nov. 5	2	0	0	0·5			0	0
6–8 November SIXTH insecticide application								
Nov. 7–Dec. 12	10	0	0	0			0	0
17–18 December SEVENTH insecticide application								
Dec. 19–Jan. 8 .	7	0	0	0			0	0
9–10 January EIGHTH insecticide application								
Jan. 12–31 .	6	0	0	0			0	0
Feb. 1963 .	5	0	0	0			0	0
Mar. 1963 .	4	0	0	0			0	0
April 1963 .	5	0	0	0			0	0
May 1963 .	4	0	0	0			0	0
June 1963 .	4	0	0	0			0	0
July 1963 .	4	0	0	0			0	0
Aug. 1963 .	3	0	0	0			0	0
Sept. 1963 .	6	0	0	0			0	0
Oct. 1963 .	3	0	0	0			0	0
Nov. 1963 .	1	1	0	0			0·2	0
Dec. 1963 .	2	0	0	0			0	0

Table III.—North Block: Mean catches of *G. morsitans* per set of fly rounds

Period 1962–3	No. sets of rounds	Old males	Old females	Tenerals m & f	Female %	Teneral %	A.D. (O.M.)
June 1962	2	5·5					2·4
July 1–7	2	6	2	1·5	25	16	1·8
8–14	3	10	3	1	23	7	3
15–21	3	9·7	3	0·3	24	2·3	2·9
22–31	3	9	2·3	0·7	20	5·8	2·7
1–4 August FIRST insecticide application							
Aug. 5–11	2	6	2	0·5	25	5·9	1·8
Aug. 12–Sept. 1	2	2	0	1	0	33	0·6
Sept. 2–7	2	1·5	1	2	40	45	0·4
8–12	2	7·5	1·5	2	17	18	2·3
13–15 September SECOND insecticide application							
Sept. 14–22	4	1	0·5	0·25	22	37	0·3
23–29	2	1	0·5	1·5			0·3
30–Oct. 6	2	1	0	0			0·3
Oct. 7–13	2	0·5	0	1·5			0·15
14–18	2	0	0	0			0
18–20 October THIRD insecticide application							
Oct. 21–27	2	0	0·5	0·5			0
28–Nov. 3	2	0	0	0			0
Nov. 4–7	1	0	0	0			0
8–10 November FOURTH insecticide application							
Nov. 11–17	2	0	0	0·5			0
18–24	2	0	0·5	0			0
25–Dec. 1	2	0	0	0·5			0
Dec. 2–8	2	0	0	0			0
9–17	3	0	0	0			0
18–20 December FIFTH insecticide application							
Dec. 21–Jan. 6 1963	5	0	0	0			0
7–8 January SIXTH insecticide application							
Jan. 9–31	6	0	0	0			0
Feb. 1963	5	0	0	0			0
Mar. 1963	4	0	0	0			0
April 1963	5	0	0	0			0
May 1963	4	0	0	0			0
June 1963	4	0	0	0			0
July 1963	5	0	0	0			0
Aug. 1963	2	0	0	0			0
Sept. 1963	4	0	0	0			0
Oct. 1963	4	0	0	0			0
Nov. 1963	2	0·5	0	0			0·15
Dec. 1963	2	0	0	0			0

Table IV.—Costing of 1962 experiments with that for 1959 for comparison. Eight full applications assumed in all cases

Head	1962 South Block		1962 North Block		1959 North Block	
	Basic	Due to locality	Basic	Due to locality	Basic	Due to locality
	£	£	£	£	£	£
Transport						
Personnel. . .	38	93	38	93	15	66
Equipment . .		99		99		50
Flying . . .	333	57	407	57	518	168
Salaries						
Pilot . . .	109	24	133	24	132	27
Ground Party . .	127		155		150	
Insecticide						
Concentrate . .	1,786	67	1,353	50	1,848	66
Diluent . . .	63	33			715	394
TOTAL . .	£2,456	£373	£2,086	£323	£3,378	£771
Cost per sq. mile . .	£224	£34	£190	£30	£307	£70
Overall cost per sq. mile	£258		£220		£370	

would be incurred wherever an experiment was carried out, those " due to locality " will be variable according to distance from railhead, etc.

The costs of biological assessment, scientific supervision and preparation of marker paths are not included because these will vary with the locality, will be less intensive in a routine operation and can be readily costed in the light of local conditions by anyone planning such an operation. The items shown have been calculated as follows:

(1) **Transport**

(i) **Personnel.** Two Land-Rovers, one staying down for the whole operation and one visiting for each application. Total: nine round trips of 330 miles. Running on site 150 miles per application for both experiments together. At Shs. 1/25 per mile. Divided equally between the two experiments.

(ii) **Equipment.** A 3-ton lorry made seven round trips of 330 miles each, charged at Shs. 1/- per ton mile. Insecticide and diesoline are costed at this rate and given separately, the residue charged to transport of equipment which included a sectional store, aviation and M.T. fuel and many miscellaneous items.

(2) **Aircraft**

(i) **Transit.** 13·3 hours divided equally.

(ii) **Spraying.** Eighty-seven hours divided between South and North in ratio 45/55. The longer runs in the South Block reduced the proportion of dead flying time over this block. Costed at £8/10/- per hour, including depreciation but not A/c insurance which could be a large item for a commercial operator, but does not apply to our aircraft.

(3) **Salaries**

(i) **Pilot.** £2,200 p.a., eight days in transit divided equally, and forty days on spraying allocated in ratio 45/55.

(ii) **Ground Party.** One field officer at £1,200 p.a. and six assistants at £12 p.w. for eight weeks, allocated 45/55.

(4) **Insecticide**

At special Government contract rates: 940 gallons Dieldrex 15T, 660 gallons 10% Telodrin solution, 470 gallons Diesoline. Actual cost of road transport allocated as due to locality. All costs are rounded up to the nearest pound.

Considering basic costs first; the North Block was less economical to spray because the shorter runs increased the proportion of flying time spent on turns. This, and the greater number of swathes, sometimes led to an extra sortie being needed to cover this block, hence the greater allocation for pilot's salary. On the other hand, the cost of insecticide is very considerably less.

The costs for 1959 are higher than those quoted in Burnett *et al.* 1961 because a charge has been included for aircraft depreciation. The basic cost per square mile is, however, little affected. Comparing 1959 and 1962, transport of personnel and equipment has risen because of the use of two Land-Rovers and the return of all senior staff to Arusha between applications. In addition, all outward and empty return journeys to Chungai have been charged to these experiments, whereas in 1959 they could often be legitimately charged to other work. In fact, some trips could fairly be charged to supervision, which is otherwise not included in these costs. Flying over the block was cheaper because of the change to a faster and more economical aircraft and to a much more concentrated insecticide. That due to locality is genuinely reduced by the change of aircraft, but further artificially cut because it is divided between two experiments, whereas in 1959 it was charged to one. The same applies to salaries, although there is again a genuine reduction because it took two to three days to cover each block instead of a minimum of four. However, salaries have risen and provision is made in the ground party for two extra unskilled assistants, while the pilot's salary now includes an hourly flying pay element that used to be included in the cost per hour for operating the aircraft. The greatest economy has been in the cost of, and heavy transport charges for, diluent, although the rate for both has increased.

DISCUSSION

The evaluation of this experiment depends on the provenance of the three flies captured in 1963. The single *G. swynnertoni* was almost certainly an immigrant. Although the combined blocks used to support a few *G. swynnertoni* in the mid-1950's (D. Turner, personal communication), Foster *et al.* (1961) found only three specimens in 1958 and none at all were captured by Burnett *et al.* (1961) in 1959–60. It is therefore likely that there was no resident and self-sustaining population but only occasional strays from elsewhere. The A.D.'s for *G. morsitans*, taken over the year, were 0·0073 and 0·0062 in the South and North Blocks respectively. At the "standard availability" of 10% (EATTRRO, 1955) these would represent populations of the order of 0·073 and 0·062 males per square mile, or of 0·22 and 0·18 flies of both sexes. Even if this estimate was an order of magnitude too small, such populations seem hardly viable or self-sustaining. Flies are known to have been living only three miles from the block at Mauno and the captures followed a known movement

of elephant. Thus there is considerable justification for assuming that the resident population was exterminated.

It is therefore possible to claim that this is the most successful experiment on the aerial control of tsetse yet made. The best previously has been that at Atta Island (Hocking, Burnett and Sell, 1954) where no tsetse were found for six months. It may be significant that Atta and Chungai have been the only two blocks adequately isolated from other infested bush, but apart from this the differences between the two operations are so many and varied that useful comparisons are few.

It is unfortunate that tsetse numbers in the South Block had been falling over recent years, even before settlement of the block started, and that settlement intensified to some extent in early 1962, because it is impossible to say that the experiment would have been so successful against a resurgent population. However, very little settlement has taken place in the part of the block always most favoured by tsetse and there was no fall in control catches during the period covered by the applications. When to this are added the interruptions suffered and the extemporised nature of the formulation, one may conclude that the method of very low volume application had a good deal of capacity in reserve in this experiment and with further development will have more, sufficient to cope with more resilient populations.

A factor which may have been of some importance is the virtual absence of escapes of fly from unsprayed into sprayed zones, where they are safe because of the lack of any residual effect of this type of application. The calculations made at various times on the effect of overlap are given in a departmental report (Burnett, 1963 a). It is estimated that the percentages of the population escaping in this way may have been 1·6–5·5 at Chungai in 1958, 1·0–3·8 in 1959 and only 0·3–1·1 in 1962.

The comparative trial of Telodrin has been of little value due to the small numbers of flies and the extensive deviation from the plan of operations. The area dosages of Telodrin and Dieldrin were in inverse ratio to their known toxicities to *G. morsitans* determined in the laboratory (Burnett, 1961 a), but the volume dosage of Telodrin solution was 50% that of the Dieldrin. However, with different formulations it is likely that the effective number of droplets was not in the same ratio. Despite the misfortunes attending the North Block experiment six applications of Telodrin were apparently as good as eight of Dieldrin in the different circumstances in which it was used and very worth-while economies were made.

The most important step in reducing costs, increasing efficiency on an economic basis, and possibly in increasing the efficacy of the insecticide expended, has been the reduction in volume dosage by 80% compared with 1959–60. This was not such a long shot as it may appear at first sight, because the dosage of active ingredient remained constant and it was known that within a few seonds of emission most of the relatively volatile kerosene in the dilute spray was lost (Yeo and Thompson, 1954), and that evaporation is fastest for the smaller drops that are the most effective in penetrating bush and impacting on tsetse flies. However, during the time required to enter the airspace beneath the forest canopy, the aerosol cloud produced directly has important differences from one of identical ultimate spectrum produced by evaporation. At canopy level wind speed falls off greatly, more especially at low speeds in the free air (Thompson, 1953; Table I). Most of the dispersal suffered by the aerosol cloud due to drift or turbulence will take place above the canopy, and the interval between emission and arrival at the canopy increases inversely as the square of the diameter is reduced e.g. the time to fall 5 feet in stable air increases

from 8 seconds for an 80μ drop to 31 and 57 seconds for drops of 40μ and 30μ respectively (Davies, 1947). These are the approximate sizes of drops containing an LD 95 for young *G. morsitans* of 2½% Dieldrin, 20% Dieldrin and 20% Telodrin respectively. In a beam wind of 3 feet per second (2 m.p.h. or 0·91 metres per second) drift will be 8, 31 and 57 yards for these droplets and the drift will increase proportionately with increased wind speed or height above the canopy. Even with a wind of 3 feet per second and emission at 5 feet, the Telodrin drop will drift a full swathe width before entering the canopy. Thus low flying, low wind speeds and stable atmosphere become even more important with more concentrated solutions. It is, however, probable that in very stable, calm conditions dispersal of the aerosol will improve with smaller droplets because they remain suspended for longer periods and this would be an important improvement (compare Burnett and Thompson, 1956, Trial Y). The occurrence of deep inversions and periods of calm beneath them at night at certain times of the year makes the development of night operations attractive. For reasons of safety it would be necessary to fly at greater heights, but the effect of this would be completely offset by the absence of wind. The main advantage gained would be a considerable increase in the period in each twenty-four hours suitable for spraying. As a routine measure this would require extra pilots per aircraft but this would lower the cost per flying hour (see Table IV). Alternatively, when conditions are too bad to operate at all in daylight it may be possible to carry on quite satisfactorily after dark.

Further improvements in methods are possible by developing the best formulation for use through the aerosol generator. It is unlikely that our extemporised Dieldrex 15T/Diesoline mixture was the best possible and it had to be emitted at a higher volume dosage than was planned. The aim must always be to obtain adequate performance at reduced cost and, because in 1962 at Chungai formulated insecticide accounted for 69% and 58% of the cost for the South and North Blocks respectively, this item offers the best chance of making large savings. The 20% Dieldrin solution used in 1959 is cheaper than Dieldrex 15T and if it is suitable for exhaust aerosol generation its use would make a considerable saving. If it is used successfully undiluted at the dosage originally planned at Chungai (0·0156 gal./acre) a total saving is possible of some £50 per square mile. First priority should be given to developing the best and most economical Dieldrin formulation. This may reduce insecticide costs to the level where Telodrin, a more toxic material to handle, offers no economic advantage over Dieldrin. However, if the limit in reduction of volume dosage has been reached with Dieldrin, Telodrin offers prospects of further progress, and so may Thiodan, a much safer material that may shortly compete economically with Dieldrin. It is already available as a 35% emulsifiable formulation.

Further reduction in volume dosage may or may not be worth while. The change from diluted Dieldrex 15T to a Dieldrin solution would give economies anyway, because the solution is a cheaper formulation. Telodrin, at half the volume dosage of the Dieldrin, certainly killed tsetse but it has not had an adequate test. Simple reduction in volume dosage can give only small economies because solvents are relatively cheap (although fairly expensive to transport) and an increase in concentration may require more expensive solvents in any case. If 20% Dieldrin is effective, the saving in using, say, 25% will be small. However, if reduced volume is accompanied by more efficient droplet distribution, considerable economies are possible in the expenditure of active ingredients, the principal single cost of the

operation. As the efficiency of the droplet spectrum is raised the proportion of insecticide expended that is really effective increases, thus a smaller amount will do as good a job and the portion hitherto wasted can be saved. This gives a genuine reduction in cost and reduces the amount of toxic substance spread over the countryside, both desirable aims. The exhaust generator should produce fewer wasteful and undesirable droplets of over 50 microns and it is in increasing the effect of droplets in the lower range that more toxic chemicals can be advantageous. The 95% lethal dose for pregnant female *G. morsitans* can be carried in a 56 micron drop of 20% Telodrin solution, whereas such a dose for 20% Dieldrin requires a droplet of 78 microns, well outside the desirable range.

It is considered that further field trials are not justified until the matter of formulation has been decided and that any reduction in the dosage of active ingredient should await the result of trials with new formulations at present dosage rates.

Some saving is still possible in the flying costs. The reduction of the swathe width from 55 to 52·5 yards was due to a misunderstanding and an adjustment was made to the emission rate in preference to re-measuring all the swathes. There seems no reason why the swathe width should not be increased to 55 yards, with a reduction of almost 5% in the amount of flying required over the block, and perhaps of more than this by cautious steps. However, a 5% reduction in insecticide expenditure would give some three times the total saving of a 5% reduction in flying costs and probably have no measurable effect on the kill of tsetse fly obtained.

Consideration might also be given to reducing the number of applications. Eight was fixed as our standard at the beginning of aerial work and it has been used in all successful experiments (summarised in Burnett, 1962) but in all these cases except the full-scale campaign in Lango, Uganda (Hocking and Yeo, 1956), at least one and frequently several of these applications have been incomplete. Certainly where only a drastic reduction in tsetse numbers is required, and not extermination, fewer applications are needed, with resultant economies. This has been demonstrated theoretically by Yeo and Simpson (1960) and in practice by Cockbill *et al.* (1963). If ever insecticides other than chlorinated hydrocarbons are used either because of resistance acquired by the fly, or because representatives of other types became relatively cheaper, it is likely that fewer applications will be needed. This is because pregnant females do not show enhanced tolerance to those representatives of the organophosphates and substituted N-methylcarbamates that have been tested (Burnett, 1961 c, 1963 b, 1963 c); the implication of this on the use of these insecticides in tsetse control are discussed in the second of these papers. Not only is Baytex (fenthion) more toxic than either Dieldrin or Telodrin to pregnant tsetse but because there is little difference in the susceptibility of old and young flies the reduction of a population should follow more closely the theoretical model of Yeo and Simpson (1960). It should be possible, with the usual reservations, to forecast the end-point from the observed mortality of old males and, quite clearly, if the breeding section of the population is suffering the same mortality as that observed and if this is of the same order as in the latest experiment, and not an unknown but certainly smaller one, the same end-point will be attained with fewer applications.

Although the blocks were usually well isolated, a few flies appear to have arrived as immigrants. Such immigration is likely to occur in any scheme unless the area sprayed is a complete tsetse belt isolated by natural barriers many miles wide. In any lesser area, it would seem that effective exploitation of an eradication

scheme must depend on human settlement at a sufficient density to change permanently the ecology of the area and make it untenable for fly. An eradication scheme must, therefore, be part of a complete development scheme for the area.

SUMMARY

Two experiments were conducted simultaneously at Chungai, in central Tanganyika, to test new aerial insecticide disseminating equipment and a new insecticide, Telodrin, for the eradication of *G. morsitans*. Two 11-square-mile blocks of bush were treated, one with 12·3% Dieldrin and one with 10% Telodrin in oils, emitted at 0·025 gallons (14 gms. A.I.) per acre and 0·012 gallons (6 gms. A.I.) per acre respectively from an exhaust aerosol-generator fitted to a Cessna 182E high-wing monoplane. Eight applications at three-week intervals were planned, but one of the Dieldrin applications was not completed and only six Telodrin treatments were made. The inter-treatment interval was extended to six weeks in some cases.

The initial populations of tsetse were lower than expected and that attacked with Telodrin was too low for conclusive assessment of results. No *G. morsitans* were found until eleven months after spraying, when two flies, which were probably immigrants, were caught, but none was found in the succeeding month. It is concluded that the original population was exterminated. Small numbers of *G. pallidipes* and *G. swynnertoni* also present were apparently eliminated. It is considered that the isolation of the blocks was important for the degree of success achieved.

The cost per square mile for Dieldrin and Telodrin respectively was £224 and £190 for basic costs plus £34 and £30 for costs due to the locality.

It is considered that there is good scope for further reductions in costs, particularly in the use of Dieldrin, and that these may make it economically competitive with Telodrin, which requires more care in handling. Telodrin requires a more conclusive trial and, if it is practicable to reduce volume dosages below those possible with Dieldrin formulations, Telodrin will require further consideration.

Acknowledgements

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REPORT ON RESULTS FROM INSECTICIDAL CONTROL PROJECTS FOR THE ERADICATION OF *GLOSSINA* IN THE SUDAN VEGETATIONAL ZONE IN NORTHERN NIGERIA

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Recommendation V of the Ninth Meeting of ISCTR at Conakry (ISCTR Report, p. 38) requests that the Committee be kept informed of the results from the eradication project on the Komadugu Gana. The report on the meeting of the Group of Experts on Regional Campaigns for Tsetse Control, Section V (p. 416) also emphasised the need for further examination. This examination has continued and the results and conclusions are described below. The situation to date has been reviewed by Mr. H. Davies, Chief Control Officer, from whose paper, to be published in the Journal of Applied Ecology, most of the information given below is drawn.

PRESENT EXTENT OF OPERATIONS

The location and timing of operations, largely confined to the flood-plains of the Komadugu Gana and Jamaari–Katagum river systems is indicated in the table below:

Year	Location	Area sprayed sq. miles	Area sprayed sq. km.	Area re-sprayed sq. miles	Area re-sprayed sq. km.
1955–56	Komadugu Gana (Chinade) . .	7	18	—	—
1956–57	Komadugu Gana	55	143	—	—
1957–58	,, ,, ...	69	179	10	26
1958–59	,, ,, ...	115	298	—	—
1959–60	,, ,, ...	122	316	12	31
1960–61	,, ,, ...	113	293	—	—
1961–62	Jamaari–Katagum–Kiyawa (Gadau) .	245	635	—	—
1962–63	,, ,, ,, (Kiyawa) .	196	508	—	—
1963–64	,, ,, ,, (Itas) .	125	324	19	49
1963–64	,, ,, ,, (Katagum) .	165	428	—	—
		1,212	3,142	41	106

Species of *Glossina* involved

In all the areas either *G. tachinoides* Westw. or this fly in combination with *G. morsitans submorsitans* Newst. were concerned. Tsetse density ranged from the very heavy, exceeding fifty *G. morsitans* per patrol mile or twenty *G. tachinoides* per man hour, to the very light.

Technique for control

Control was achieved by the ground application of a water suspension of DDT wettable powder from pressure knapsack sprays. The concentration of actual DDT has varied from 5 to 2·5%, the latter giving as good a result as the former. The

application was restricted to the hot season, hot time of day tsetse resting sites on the woody vegetation and as experience was gained the application became progressively more selective. The final system of control evolved has been described by Davies as follows:

(*a*) The woody vegetation of all drainage lines within the flood-plain boundary is sprayed provided it appears as if it could harbour tsetse irrespective of whether any flies are caught.

(*b*) Woody vegetation is not sprayed in drainage lines which extend outside the flood-plain unless flies are actually caught there, but in more southerly latitudes such areas may be sprayed if the vegetation looks as if it provides a suitable habitat.

(*c*) All suitable vegetation between a main river and the adjacent motor tracks (put in on each side to facilitate reclamation) is treated whatever the density of tsetse in it. The distance between rivers and tracks is usually 30–100 yards but on the inside of a meander of the river it may be half a mile or more.

(*d*) On subsidiary water courses where the woody vegetation is wide, spraying is confined to a distance of about 30 yards from the channel on each bank.

(*e*) On higher ground, spraying is confined to evergreen forest islands which look as if they could harbour *G. morsitans* in the dry season. Clump thickets are not sprayed, but if tsetse are present and there are no forest islands in the vicinity, larger thickets are sprayed to provide a source of residual insecticide in the locality.

(*f*) Denser flood-plain boundary vegetation is sprayed in more southerly areas, but not in the north.

(*g*) All the above vegetation is sprayed selectively. Insecticide is applied to all sides of each tree trunk only if it is in heavy shade and is larger than 9 inches in diameter. In bush likely to be infested by *G. tachinoides* the woody stems in the lower, denser vegetation are also sprayed.

(*h*) In *G. tachinoides* areas where *G. morsitans* is absent or present in low density all spraying is confined to a height of about 2 feet above ground level; in *G. morsitans* bush where density is heavy, trunk spraying is increased to about 5 feet.

(*i*) The leafy parts of the vegetation are ignored, spraying is confined to the woody parts.

Methods used to confirm control results

The weakness in insecticidal control is that the habitat remains unchanged and re-infestation can occur after the effectiveness of the insecticide has declined, whether by invasion from outside the area or from undetected foci inside it.

The pre-spray nature and distribution of the *Glossina* population during the earlier years were studied by orthodox fly rounds which continued in some areas after spraying for a period of several months, on some occasions to be resumed at a later date for a further period. It is physically impossible to cover an area of 1,212 square miles (or 3,142 square kilometres) completely with orthodox fly rounds, and experience has shown that residual tsetse populations, if they exist in very low density, can be more readily located by special search parties directing their attention to likely areas using special methods. The whole of the reclamation area has been subjected to repeated annual searches, the self-contained survey

parties being based on tractors and trailers with tent accommodation, and moving over the whole area. It has been found that, in order to detect *G. tachinoides* in low density, it is necessary to spend four to seven days in one locality before one specimen can be caught.

Result on the Komadugu Gana (481 square miles)

Only this project has been completed sufficiently long to enable any firm conclusion to be reached, and it is from this area that additional information was requested at the Eighth Meeting of ISCTR.

It is necessary first to refer to the topography of the area, since operations were spread over a period of six years, the first application of insecticide being in 1955–6 (see map).

There is no outside source of *G. morsitans* within 40 miles of the project area, but the southern end is contiguous with permanent riverine infestations of *G. tachinoides* from which it is isolated to the south by a cleared barrier 1½ miles long.

The stream shown extending into the area from the west is mostly devoid of suitable *G. tachinoides* habitats so cannot be cleared, since there is nothing to clear. It was suspected to be a means of re-infestation by *G. tachinoides* and was included in the insecticidal applications carried out by the Ministry of Health. There is a natural barrier at Bulkachuwa and a further artificially cleared barrier at Udubo 1 mile wide.

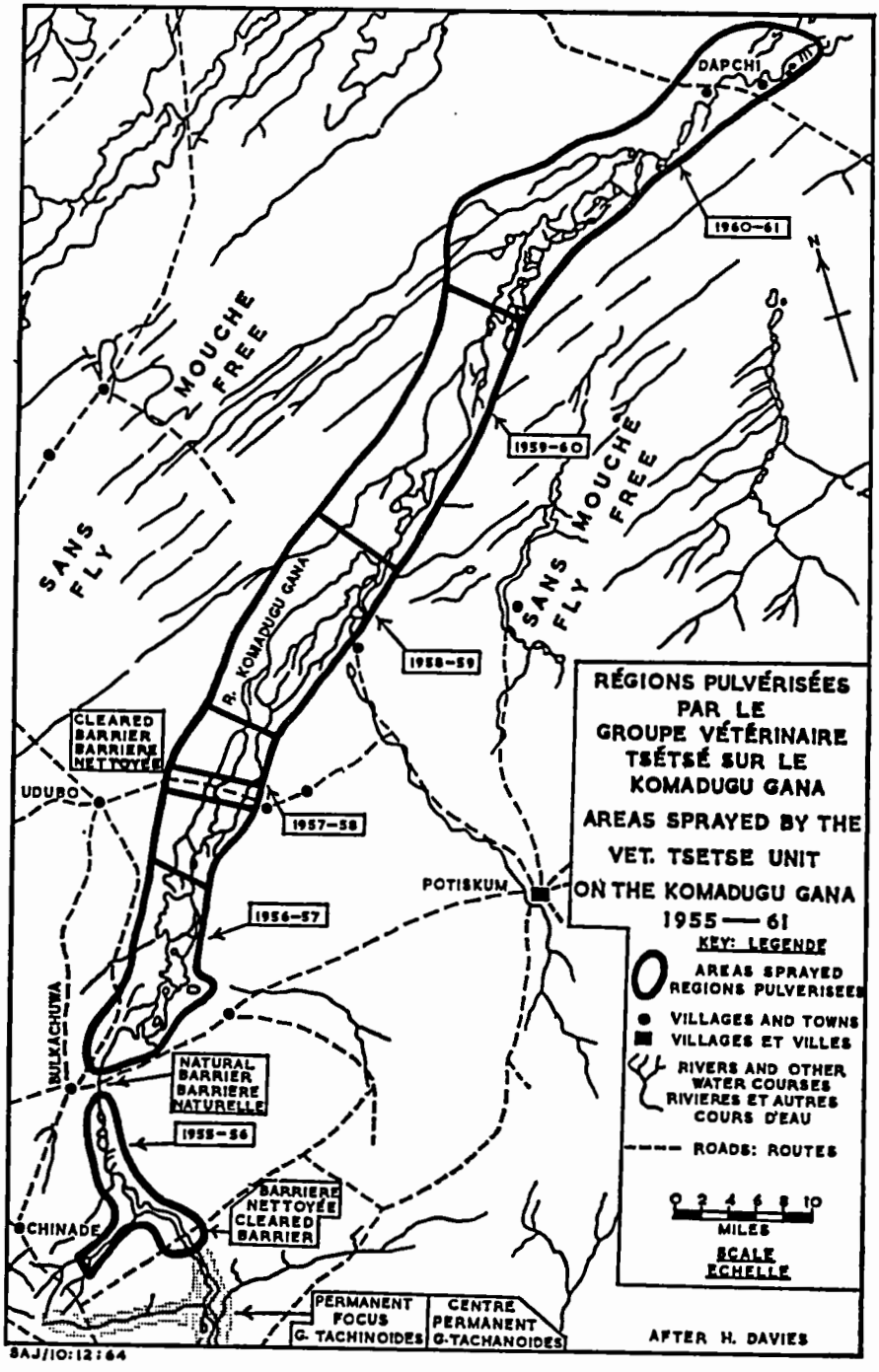
The 1955–6 area has been surveyed at least once, sometimes twice or even three times annually, every year up to 1964. In addition, fly rounds have been followed periodically over much of it.

This original reclamation area was the site of the pilot scheme from which the rest of the project grew; at the time it was undertaken the control principles used were new and in the formative stage and, being a veterinary project, the main emphasis was on the control of *G. morsitans*. Results exceeded expectations and *G. tachinoides* was also eliminated. The further control of the latter species which, unlike the former, had a continuous distribution up-stream to the south was undertaken by the Sleeping Sickness Service of the Ministry of Health who sprayed out the southern part of the area, beyond the *G. morsitans* limit which was the boundary of the initial Veterinary Project, and who put in the cleared barrier.

To date not a single individual of *G. morsitans* has been found subsequent to the initial application of insecticide in 1955–6. The control of *G. tachinoides* proved more difficult, probably because of re-infestation along the tributary from the west or across the barrier, and several applications of insecticide were made, the last one in April 1961. Since that date regular fly rounds and special searches have located the occasional *G. tachinoides* in the southern part of the reclamation area. A series of fly rounds covering the reclamation area and the nearest infested stream outside gave the following results over a period of seven months.

Total *G. tachinoides* caught

Inside reclamation area		Outside reclamation area	
Times patrolled	Catch	Times patrolled	Catch
102	2	26	185


DAPCHI
1960–61
1959–60
1958–59
1957–58
1956–57
1955–56
MOUCHE FREE
SANS FLY
R. KOMADUGU GANA
CLEARED BARRIER
BARRIERE NETTOYEE
UDUBO
POTISKUM
NATURAL BARRIER
BARRIERE NATURELLE
BARRIERE NETTOYEE
CLEARED BARRIER
CHINADE
PERMANENT FOCUS G. TACHINOIDES
CENTRE PERMANENT G. TACHANOIDES
RÉGIONS PULVÉRISÉES PAR LE GROUPE VÉTÉRINAIRE TSÉTSÉ SUR LE KOMADUGU GANA
AREAS SPRAYED BY THE VET. TSETSE UNIT ON THE KOMADUGU GANA 1955 — 61
KEY: LEGENDE
AREAS SPRAYED REGIONS PULVERISEES
VILLAGES AND TOWNS
VILLAGES ET VILLES
RIVERS AND OTHER WATER COURSES RIVIERES ET AUTRES COURS D'EAU
ROADS: ROUTES
0 2 4 6 8 10
MILES
SCALE ECHELLE
AFTER H. DAVIES
BAJ/10:12:64

The special searches which have been conducted have also yielded occasional solitary individuals of *G. tachinoides*; for instance, a team of ten experienced fly catchers can sometimes catch a single specimen if they search the area for a week.

With regard to the remainder of the Komadugu Gana area (474 square miles, 1956–61) no *Glossina* at all have been caught in the reclamation area more than six weeks after the application of insecticide with the notable exception of a solitary specimen of *G. tachinoides* and five individuals of *G. morsitans* as late as five months after insecticide was applied in early 1957. No further application of insecticide was made to deal with these flies and no further specimens could be found despite repeated searches. Since then all the reclaimed area, which was increasing annually, has been surveyed each wet season up to 1962 and during three separate dry seasons. The last comprehensive survey took place in the dry season of 1962–3, when the Veterinary Tsetse Unit survey team was accompanied by a Ministry of Health survey team experienced in the detection of *G. tachinoides* in low density. The combined team spent five months searching the flood-plains and was unable to catch a single specimen of *Glossina*. During the dry season of 1963–4, the 1956–7 sprayed area was intensively surveyed and again during the rains of 1964. No specimens of *Glossina* can be found.

DISCUSSION ON THE RESULTS ON THE KOMADUGU GANA

When faced with the task of searching for small flies in low density over areas of 500 square miles it is not easy at first to be confident that *Glossina* could not be overlooked. However, it has been shown that the methods used can detect *Glossina* even in very low density, as can be seen from the results of the examination of the 1955–6 area. We have also had experience on the adjacent, but quite distinct, Jamaari–Katagum river where re-invasion of the reclamation area occurred from a nearby untreated focus. This experience demonstrates that when such a thing is happening it is detected by the methods in use (see later).

One must also consider the time factor. The southernmost area was first treated with insecticide in 1955–6. Since that date no insecticide applications have been made to control *G. morsitans*. The last application for the control of *G. tachinoides* was made to part only of this area in April 1961. For the rest of the Komadugu Gana project area no insecticide has been applied since the dates as noted on the map, which refer to the period of December, January, February, March and April of the years given. The habitat throughout is ideally suited to both species of flies, the 1956–7 area being particularly so. It would be reasonable to expect that had any viable *Glossina* population survived the original insecticide application the flies would now be present in some density.

A further point to be considered is the persistence of DDT wettable powder on bark in this area. The problem has been the subject of field studies by Mr. M. G. Cook, of the Veterinary Tsetse Unit, who has clearly shown that the first indication of a decline in effectiveness does not occur until ninety-seven days have elapsed. Even after 139 days the corrected mortality after exposing test *Glossina* for only one minute ranged from 50% to 80% within forty-eight hours of exposure. Evidence has been acquired here which tends to confirm the work of Dr. Baldry at WAITR, who has shown that insecticide deposits may be active after twelve months and that though activity may wane through the dry season it revives for a period with the onset of the rains.

As yet *G. tachinoides* only exists in exceptionally low density in part of the 1955–6 area where previously they had been dense. Even after 3½ years they are detected with difficulty. The precise status of this fly population, whether in fact it is self-contained or still dependent on immigrants, is not certainly known but the events described suggest the extermination of the original population. The occasional individuals which can now be found are likely to be immigrants from contiguous infestations not yet controlled, since where no contiguous infestation exists further north, no *G. tachinoides* can be found. Control measures directed at the source of re-invasion may establish this important fact. The whole of this reclamation area now holds several thousand head of cattle where previously none dare take his livestock. There have been no complaints of trypanosomiasis.

Results on the Jamaari–Katagum river (731 square miles)

Work has continued over the period 1961–4. It is therefore much too early to come to any definite conclusion, though experiences here have a bearing on the assessment of the Komadugu Gana results.

Survey checks and fly rounds covering the southern part of the 1961–2 area (Gadau, 245 square miles) revealed a few individuals of *G. morsitans* and one specimen of *G. tachinoides* associated with a path and cattle track from the north, but intensive surveys over the rest of the area extending north-eastwards failed to find any flies in the remaining two-thirds of this area. The source of these flies was later traced to an untreated focus 4 miles away traversed by a well-trodden cattle track. The source of this infestation was subsequently included in the spraying for 1963–4 (Itas) and involved a re-spray of 19 square miles of the 1961–2 area (Gadau).

This experience indicates that the persistence of flies, which when it occurred was detected by the methods in use, was not due to a failure in technique in the area involved but was the result of re-invasion from an untreated focus.

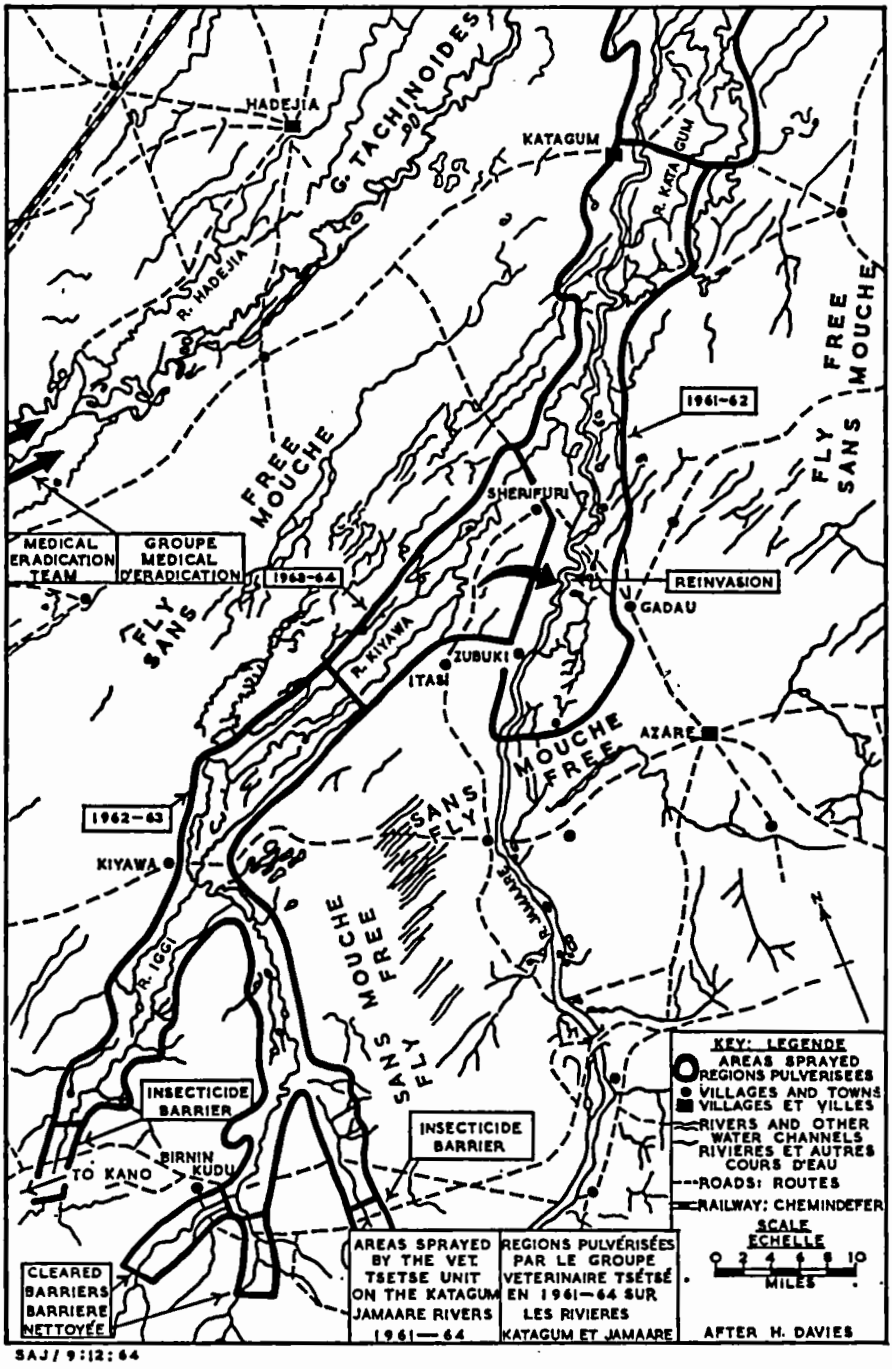
Near the southern end of the 1962–3 area (Kiyawa) which had been surveyed twice apart from the usual routine checks which are made in the rear of the eradication team, one *G. morsitans* was caught late in 1963 and another one in November, that is five and seven months after the application of insecticide. These flies may have eventually expired but no risk was taken and the localities were re-sprayed in April 1964.

It is much too early to come to any conclusions regarding a final result in the Jamaari–Katagum river area. The system is parallel to but 35 miles distant from the Komadugu Gana. The experiences here are quoted to illustrate that the methods in use do detect *Glossina* in very low density. When the lapse of time is also considered these findings give greater confidence in the validity of the continuing negative result on the Komadugu Gana.

The complete isolation and consolidation of the southern ends of the Komadugu Gana and Jamaari–Katagum river project areas is not yet secured and will to some extent depend on the future extension of tsetse reclamation work in Northern Nigeria. In the meantime the situation is under continuing observation.

CONCLUSION REGARDING THE KOMADUGU GANA

G. morsitans and *G. tachinoides* have been exterminated, as judged by current methods of detection throughout the reclamation area. The 1955–6 area is subject to infiltration by *G. tachinoides* from contiguous infestations nearby. Though these


HADEJIA
G. TACHINOIDES
KATAGUM
R. KATAGUM
R. HADEJIA
FREE MOUCHE
FLY SANS
1961-62
SHERIFURI
REINVASION
GADAU
MEDICAL ERADICATION TEAM
GROUPE MEDICAL D'ERADICATION
1963-64
R. KIYAWA
ZUBUKI
ITAS
AZARE
1962-63
KIYAWA
R. IGGI
R. JAMAARE
INSECTICIDE BARRIER
BIRNIN KUDU
TO KANO
CLEARED BARRIERS BARRIERE NETTOYÉE
AREAS SPRAYED BY THE VET TSETSE UNIT ON THE KATAGUM JAMAARE RIVERS 1961—64
REGIONS PULVÉRISÉES PAR LE GROUPE VETERINAIRE TSÉTSÉ EN 1961—64 SUR LES RIVIERES KATAGUM ET JAMAARE
KEY: LEGENDE
AREAS SPRAYED REGIONS PULVERISEES
VILLAGES AND TOWNS VILLAGES ET VILLES
RIVERS AND OTHER WATER CHANNELS RIVIERES ET AUTRES COURS D'EAU
ROADS: ROUTES
RAILWAY: CHEMINDEFER
SCALE ECHELLE
0 2 4 6 8 10
MILES
AFTER H. DAVIES

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flies could re-establish themselves in normal density in the course of time the situation can be controlled.

The economic benefits deriving from this work, both as a result of the reclamation of valuable grazing now used by many thousands of head of cattle, the opening of fertile farming areas, and the removal of the cause of infection in an area which has been an endemic focus of human sleeping sickness exhibiting periodic severe epidemics the last of which occurred as recently as 1955, have been very great. The Northern Nigeria Ministry of Animal and Forest Resources has confidence in the result and the objective remains the complete extermination of *Glossina* in the project areas.

Acknowledgements

Acknowledgements are due principally to Mr. Howell Davies who has supervised much of the work described and on whose recent review of the situation much of this report is based.

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ETUDE DE L'EFFET DE L'HCH NEBULISE SUR UNE POPULATION DE *GLOSSINA PALPALIS GAMBIENSIS* VANDERPLANK 1949, DANS UNE GALERIE FORESTIERE (KANKALABA, REPUBLIQUE DE HAUTE-VOLTA)

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INTRODUCTION

La pulvérisation d'un insecticide rémanent sur la végétation des galeries forestières constitue la technique la plus sûre et la moins onéreuse pour éliminer les espèces de glossines riveraines.

L'application d'un insecticide par nébulisation n'a pas fait l'objet d'études systématiques. Fairclough (1956) a utilisé le Swingfog pour désinsectiser les trains qui traversent une zone infestée de *Glossina longipennis* Corti, sur la ligne Mombassa–Nairobi. Le DDT fut employé en solution dans le kérosène. L'auteur conclut que le " fogging " présente un effet réel mais que le coût de l'opération est plus élevé qu'un contrôle thérapeutique.

Au Nyassaland, Steele (1956) a utilisé le Swingfog pour tenter d'éliminer *G. brevipalpis* Newstead d'un bois. L'auteur précise qu'il faut employer une solution de 4% d'isomère gamma de l'HCH, en mélange avec de l'huile diesel pour assurer une mort rapide des glossines dans des cages en toile métallique, à 27 mètres de la machine. Il y eut six applications à dix jours d'intervalle. Aucune mouche n'était aperçue le jour du traitement, quelques unes apparaissaient quatre jours plus tard.

La technique de la nébulisation (fogging) connaît quelque défaveur en raison des difficultés d'opérations.

La faible rémanence des produits nébulisés nécessite plusieurs traitements. Les intervalles entre les traitements sont imposés par la durée du premier cycle ovarien de l'espèce à éliminer et l'action de l'ensemble des traitements doit couvrir une période au moins égale à la durée maxima du stade pupal. En effet, si tous les adultes sont tués en un jour ou deux, une population nouvelle va se reconstituer à partir du stock de pupes déposées avant le premier traitement.

On ne peut faire fonctionner les appareils que tôt le matin, avant le lever du soleil et le soir après le coucher. Dans la journée, le nuage insecticide est dispersé par les mouvements de l'air. On peut cependant opérer par temps couvert.

Malgré les inconvénients inhérents à la technique il semble qu'une étude plus approfondie soit nécessaire pour connaître les possibilités d'emploi dans le traitement de petites galeries forestières.

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I. — CONDITIONS DE L'EXPERIMENTATION

Les essais de nébulisation ont été réalisés dans une galerie forestière près du village Sénoufo de Kankalaba (10°45′ Nord — 5°17′ Ouest), en République de Haute-Volta (voir carte schématique — Fig. 1).

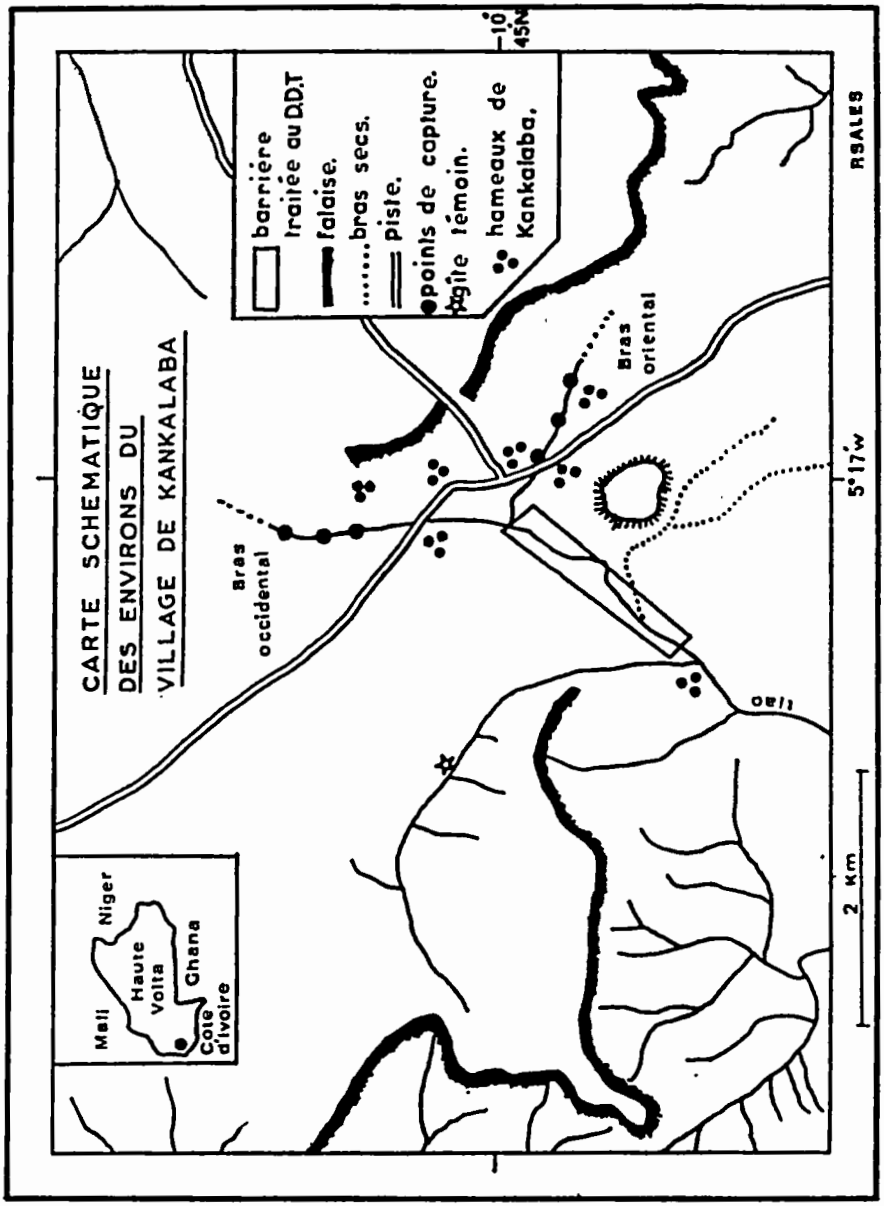

FIG. 1

Le village est situé en zone de savane boisée (aires septentrionales (17) à *Isoberlinia doka* et *I. dalzielii in*: Carte de la végétation de l'Afrique au sud du tropique du Cancer (Aubreville et coll., 1958)), près des sources du Tiao. Cet

affluent du fleuve Leraba (Comoé) coule du nord vers le sud en traversant une région accidentée. Nous conviendrons d'appeler " bras occidental " et " bras oriental " les deux bras qui entourent le village et dont le confluent se trouve à deux cents mètres au sud de ce dernier.

Les sources sont situées au fond de trous assez profonds près d'une falaise qui limite un relief tabulaire au nord.

Le lit, d'abord très encaissé sur deux cents mètres, s'élargit et dessine des méandres après le confluent des deux bras.

La galerie forestière est de largeur variable (de 0 à 40 mètres). Elle est constituée de grands arbres à canopée élevée sous laquelle croissent une végétation arbustive, des buissons et des lianes. En certains endroits les arbres ont été abattus et en d'autres les rives sont nues et bordées de jardins.

Sur chacun des bras se trouvent cinq à six points d'eau utilisés par les habitants du village de Kankalaba pour se ravitailler. La faune de la galerie et de ses environs comprend des varans, des antilopes, des singes et des crocodiles. Les troupeaux du village viennent s'abreuver au marigot.

Le village est composé de plusieurs hameaux dont certains sont situés à moins de cent mètres des points d'eau. Le contact homme-glossines est donc très étroit. En 1962, plus de cent nouveaux trypanosomés ont été dépistés.

II. — TECHNIQUE

1. — Appareil

Deux appareils nébulisateurs du type " Swingfog SN 7 " étaient employés à tour de rôle pour éviter un échauffement excessif; ils sont munis d'un réservoir de produit d'une capacité de 4,2 litres et d'un réservoir d'essence de 1,3 litres. La mise en marche est assurée par une magnéto. Des gicleurs permettent de régler le débit du mélange insecticide.

2. — Progression de l'équipe

En avant de l'appareil, un manœuvre ouvre la marche pour frayer un passage et sonder la profondeur du marigot; vient ensuite le porteur de l'appareil dirigé par un chef d'équipe (pour les essais: un technicien). En lisière de la galerie, marchent les porteurs des réserves de produit et d'essence et le porteur du second appareil. Le personnel peut donc être réduit à six personnes. La vitesse de progression peut atteindre 1 km/heure.

3. — Dispersion du nuage

Nous avons traité en marchant de l'aval vers l'amont car il règne un léger courant d'air au fond du lit, au-dessus de l'eau. Le nuage est parfois entraîné à plusieurs centaines de mètres du point d'émission. Le produit sort de l'appareil en un jet assez court que l'on dirige vers le sol; il subit ensuite un mouvement ascendant avant de se rabattre en s'étalant sur toute la largeur de la galerie. Dans les parties de celle-ci où la végétation est peu dense, le vent peut disperser le nuage dans la savane voisine.

Les heures d'utilisation de l'appareil étaient de 6 heures 30 à 7 heures et de 17 heures 30 à 19 heures.

Tableau I. — Effectifs des captures de *G. palpalis gambiensis* Vanderplank au gîte témoin (2 captureurs placés en un point d'un marigot tributaire du Tiao, près de Kankalaba, République de Haute-Volta)
Période du 9 janvier au 9 avril 1964

Date	Mâles			Femelles				Total
	T	V	Total	T	N	P	Total	
9.1.64	1	10	11	—	3	5	8	19
10.1.64	0	8	8	—	2	2	4	12
18.1.64	2	16	18	—	6	6	12	30
24.1.64	1	11	12	1	2	2	5	17
7.2.64	3	23	26	2	6	14	22	48
18.2.64	1	8	9	—	3	11	14	23
19.2.64	2	12	14	—	3	7	10	24
20.2.64	4	25	29	4	4	3	11	40
21.2.64	0	14	14	2	3	5	10	24
26.2.64	3	17	20	0	0	3	3	23
27.2.64	1	10	11	1	2	8	11	22
4.3.64	0	11	11	0	1	2	3	14
5.3.64	0	6	6	0	1	0	1	7
6.3.64	1	12	13	1	2	4	7	20
12.3.64	0	11	11	1	1	3	5	16
13.3.64	0	7	7	0	0	3	3	10
18.3.64	0	7	7	1	1	7	9	16
19.3.64	2	9	11	0	2	5	7	18
20.3.64	0	9	9	0	4	3	7	16
25.3.64	0	4	4	0	3	2	5	9
26.3.64	1	6	7	0	2	3	5	12
1.4.64	0	8	8	1	0	2	3	11
2.4.64	3	11	14	2	0	5	7	21
3.4.64	0	6	6	0	3	2	5	11
8.4.64	0	5	5	0	0	0	0	5
9.4.64	0	6	6	0	2	2	4	10

T = glossine " ténérale " N = nullipare
V = mâle non " ténéral " P = pare

4. — Les traitements

L'HCH a été choisi en raison de son coût assez bas et de sa faible toxicité pour l'homme par rapport à la Dieldrine. Le produit commercial utilisé est le " Procidacri 100 " (100 grammes d'isomère gamma du HCH (Hexachlorocyclohexane) par litre de concentré).

Six traitements ont été appliqués aux dates suivantes: 17 janvier, 5 février, 20 février, 5 mars, 20 mars et 3 avril.

Le premier traitement a consisté en une nébulisation d'un mélange d'une partie d'HCH pour deux parties de distillat de diesel. L'appareil était muni du gicleur donnant le plus faible débit horaire (10,5 litres de mélange). La concentration était de 3,3% (P/V) d'isomère gamma. La consommation totale a été de 27 litres, soit 9 litres d'HCH (900 gr. d'isomère gamma). Pour le deuxième traitement le mélange a éte de deux parties d'HCH pour une partie de distillat de diesel; le débit n'a pas été modifié. La consommation totale a été de 20 litres de mélange (13 litres d'HCH); la concentration était de 6,6% (P/V) d'isomère gamma. Comme ce dernier traitement était encore insuffisant pour éliminer les glossines adultes la concentration du mélange a été maintenue mais le débit horaire a été porté à 30 litres. La consommation totale a été de 35 litres (23 litres d'HCH). Ce traitement ayant été efficace nous l'avons maintenu lors des trois applications suivantes.

Tableau II. — Effectifs comparés des captures de *G. palpalis gambiensis* Vanderplank en trois points du bras occidental du marigot Tiao (village de Kankalaba, République de Haute-Volta)

Période du 17 décembre 1963 au 10 avril 1964

Catégories d'âge — T: mâle ténéral
V: vieux mâle (non ténéral)
Nt: femelle nullipare et ténérale
N: femelle nullipare non ténérale
P: femelle pare

Le signe + signifie que des glossines ont été aperçues mais n'ont pas été capturées
Le tiret — signifie que la catégorie n'a pas été déterminée

Dates	Source					Gué 1					Gué 2					Totaux des différentes catégories pour les 3 points de capture					
	Mâle		Femelle			Mâle		Femelle			Mâle		Femelle			Mâle		Femelle			Mâle + Femelle toutes catég.
	T	V	Nt	N	P	T	V	Nt	N	P	T	V	Nt	N	P	T	V	Nt	N	P	
17.12.63 .	—	7	—	—	1	9	—	8	—	—	1	6	—	11	—	—	0	6	—	26	—	—	2	21	49
18.12.63 .	—	36	—	—	1	17	—	15	—	—	1	15	—	24	—	—	3	16	—	75	—	—	5	48	128
9. 1.64 .	0		27	—	1	10	2		19	—	2	11	0		18	—	0	2	2		64	—	3	23	92
10. 1.64 .	1		14	—	2	10	0		10	—	0	6	3		7	—	0	6	4		31	—	2	22	59
1er traitement le 15 et 16 (1 partie d'HCH pour 2 parties de distillat de diesel ; débit horaire théorique de l'appareil : 10,5 litres)																					
16. 1.64 .	—		—	—	—	0		5	—	1	0			+					—		5	—	1	0	6
17. 1.64 .	1		2	—	2	14	1		1	—	0	0	0		1	—	0	0	2		4	—	2	14	22
18. 1.64 .	0		6	—	4	15	0		2	—	2	6	0		8	—	3	1	0		16	—	9	22	47
19. 1.64 .	1		6	—	3	14	1		0	—	1	1	2		3	—	7	5	4		9	—	11	20	44
24. 1.64 .	2		6	—	6	6	—		—	—	—	—	0		2	2	3	1	2		8	2	9	7	28
4. 2.64 .	2		16	1	2	2	1		14	3	1	0	1		3	0	1	3	4		33	4	4	5	50
2e traitement le 5 (2 parties d'HCH pour 1 partie de distillat de diesel ; débit horaire théorique de l'appareil : 10,5 litres)																					
6. 2.64 .	0		1	0	0	0			+				0		0	0	0	1	0		1	0	0	1	2
18. 2.64 .	0		0	0	0	0	1		1	0	1	0	1		0	0	0	0	2		1	0	1	0	4
19. 2.64 .	0		1	0	0	0	0		2	0	0	0	0		0	0	0	0	0		3	0	0	0	3
20. 2.64 .	0		2	1	0	0	2		3	1	1	1	2		3	1	0	0	4		8	3	1	1	17
3e traitement le 20 (2 parties d'HCH pour 1 partie de distillat de diesel ; débit horaire théorique de l'appareil : 35 litres)																					
21. 2.64 .																									0
22. 2.64 .																									0
26. 2.64 .			+				2		0										2		0				2
27. 2.64 .							2		0	2	0	0				1	0	0	2		0	3	0	0	5
28. 2.64 .			+				3		0				1		0				4						4
3. 3.64 .							3		1	1	1	0				0	1	0	3		1	1	2	0	7
4. 3.64 .	1		0	2	0	0										0	1	0	1		0	2	1	0	4
5. 3.64 .	1		2	0	0	1	1		1	0	2	1			+				2		3	0	2	2	9
4e traitement le 5 (idem 3e traitement)																					
6. 3.64 .															+										+
7. 3.64 .																									0
11. 3.64 .																									0
12. 3.64 .				1 femelle(?)																		1 femelle			1
13. 3.64 .			+																						+
18. 3.64 .				0	1	0																0	1	0	1
19. 3.64 .															+										+
5e traitement le 20 (idem 3e traitement)																					
20. 3.64 .																									0
21. 3.64 .																									0
25. 3.64 .			+																						+
26. 3.64 .																									0
27. 3.64 .																									0
1. 4.64 .																									0
6e traitement le 1er (idem 3e traitement)																					
2. 4.64 .																									0
3. 4.64 .																									0
8. 4.64 .																									0
9. 4.64 .																									0
10. 4.64 .																									0

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Tableau III. — Effectifs comparés des captures de *G. palpalis gambiensis* Vanderplank en 3 points du bras oriental du marigot Tiao (village de Kankalaba, Haute-Volta)

Période du 17 décembre 1963 au 10 avril 1964

Catégories d'âge — T: mâle ténéral
V: vieux mâle (non ténéral)
Nt: femelle nullipare et ténérale
N: femelle nullipare non ténérale
P: femelle pare

Le signe + signifie que des glossines ont été aperçues mais n'ont pas été capturées
Le signe '( )' signifie captures l'aprés-midi

Dates	Gué					2e Point					En amont du pont					Totaux des différentes catégories pour les 3 points de capture					
	Mâle		Femelle			Mâle		Femelle			Mâle		Femelle			Mâle		Femelle			Mâle + Femelle toutes catég.
	T	V	Nt	N	P	T	V	Nt	N	P	T	V	Nt	N	P	T	V	Nt	N	P	
11. 1.64 .		(7)		(3)			(13)		(5)			(20)		(9)			(40)		(17)			57
1er traitement le 17 (1 partie d'HCH pour 2 parties de distillat de diesel ; débit horaire théorique de l'appareil : 10,5 litres)																					
21. 1.64 .			0	0	3	1	2						0	1	1	1	2	0	1	4	8
22. 1.64 .	1	0						0	0	1			0	0	2	1	0	0	0	3	4
23. 1.64 .						1	1	3	3	2			0	1	1	1	1	3	4	3	12
5. 2.64 .	1	4	0	3	2	3	6	1	0	1	0	1	0	1	1	4	11	1	4	4	24
2e traitement le 6 (2 parties d'HCH pour 1 partie de distillat de diesel ; débit horaire théorique de l'appareil : 10,5 litres)																					
7. 2.64 .			0	0	1		+						0	0	1	0	0	0	0	2	2
18. 2.64 .	1	0						0	2	0						1	0	0	2	0	3
19. 2.64 .	0	2	0	0	1			0	1	0	1	0				1	2	0	1	1	5
20. 2.64 .	0	1	0	1	0	0	3	0	0	1			0	1	0	0	4	0	2	1	7
21. 2.64 .	0	1	2	0	0			0	0	1			1	0	0	0	1	3	0	1	5
3e traitement le 21 (2 parties d'HCH pour 1 partie de distillat de diesel ; débit horaire théorique de l'appareil : 10,5 litres)																					
22. 2.64 .																					0
26. 2.64 .	1	0					+									1					1
27. 2.64 .			+				+				2	0	1	1	0	2	0	1	1	0	4
28. 2.64 .	1	1				1	0	1	0	0			1	0	0	2	1	2	0	0	5
3. 3.64 .			+				+														+
4. 3.64 .								0	1	0						0	0	0	1	0	1
5. 3.64 .			+									+									+
6. 3.64 .																					0
4e traitement le 6 (idem 3e traitement)																					
7. 3.64 .													1		0			1		0	1
11. 3.64 .																					0
12. 3.64 .			+									+									+
13. 3.64 .			0	1	0																1
18. 3.64 .							+					+									+
19. 3.64 .							+					+									+
20. 3.64 .							+					+									+
5e traitement le 20 (idem 3e traitement)																					
21. 3.64 .																					0
25. 3.64 .			+																		+
26. 3.64 .							+														+
27. 3.64 .																					0
1. 4.64 .																					0
2. 4.64 .																					0
6e traitement le 3 (idem 3e traitement)																					
3. 4.64 .																					0
4. 4.64 .																					0
8. 4.64 .																					0
9. 4.64 .																					0
10. 4.64 .																					0

5. — Intervalles entre les traitements

L'intervalle entre les deux premiers traitements a été de vingt jours mais il a été de quinze jours entre les autres car le cycle ovarien doit être assez court en saison sèche. Mellanby (1936) a observé un cycle de 17 jours à 24° C.

6. — Barrière

Une barrière située en amont du confluent des 2 bras du marigot et longue de 1500 mètres a été traitée au DDT (3%) toutes les 3 semaines, jusqu'au 26 août.

III. — METHODE D'ENQUETE

Pour étudier l'efficacité de la nébulisation, des captures standards ont été effectuées avant le premier traitement, entre les traitements, et pendant quatre mois après le dernier traitement.

Les équipes de capture comprenaient chacune deux captureurs qui ont été postés en des points fixes de 7 heures du matin à 14 heures.

Sept points ont été choisis (voir la carte): 1 sur les bras " témoin ", 3 sur le " bras occidental " (source, gué 1, gué 2) et 3 sur le bras oriental (gué, 2e point, en amont d'un pont.)

Les glossines capturées ont été disséquées pour déterminer l'âge physiologique. Les mâles ont été classés en " ténéraux " et " vieux " (non ténéraux). L'état ténéral chez une glossine consiste en un tégument mou; le ptilinum peut faire saillie quand on comprime la tête (Buxton, 1955). Jackson (1946) a observé que les femelles demeurent ténérales jusque vers le 5e jour. Saunders (1962) classe les ténérales dans le groupe d'âge Oa (0–4 jours). Les groupes d'âge adoptés dans le présent travail sont ceux de Saunders (1960, 1962). Saunders (1960), chez *G. morsitans* Westwood et Mellanby (1937), chez *G. palpalis* (R.-D.) placent l'ovulation le 8e jour et le dépôt de la première larve entre le 17e et le 20e jour.

Les groupes ont été désignés selon la méthode de Saunders (1960) que nous rappelons brièvement:

Nullipares: Oa (1–4 jours); l'ovocyte le plus grand a moins de 0,6 mm.
Ob (4–8 jours); l'ovocyte le plus grand a plus de 0,6 mm.

Pares: Ia = un sac folliculaire et un œuf dans l'utérus (8–11 jours).
Ib = un sac folliculaire et une petite larve dans l'utérus (13–16 jours).
Ic = un sac folliculaire et une larve de troisième stade dans l'utérus (16–19 jours).
II = 2 sacs; III = 3 sacs, etc.

IV. — RESULTATS

Les résultats ont été consignés par points de capture dans les tableaux numérotés de I à VI. Le tableau synoptique VII groupe les effectifs moyens des captures totales pour des périodes de cinq jours. Les tableaux VIII–IX et X concernent l'âge physiologique des femelles. Les valeurs du tableau VII ont été reportées sur un graphique à échelle logarithmique en ordonnées (Fig. 2).

La population de glossines n'a cessé de décroître jusqu'au mois de mai (voir le graphique) pour s'accroître ensuite. Cette variation saisonnière est caractéristique de *G. palpalis* (R.-D.) en Afrique de l'ouest (Nash et Page, 1953).

Gîte traité

a) **Après le premier traitement** (2 parties de distillat de diesel pour une partie d'HCH, débit: 10,5 litres/heure). Six glossines ont été capturées sur le bras occidental et 2 sur le bras oriental. Ces individus n'étaient pas ténérales et l'on peut déduire de l'âge physiologique des femelles qu'elles avaient éclos avant le traitement. Pendant les 20 jours qui ont suivi le traitement la population s'est reconstituée. La veille du second traitement l'effectif des captures atteint 50 sur le bras occidental et 24 sur le bras oriental.

Avant le 1er traitement la proportion des glossines nullipares est de 9% au gîte occidental et 10% au gîte oriental. Après le traitement nous observons respectivement: 40% et 48%.

Tableau IV. — Effectifs des captures de *G. palpalis gambiensis* Vanderplank au gîte témoin
Période de contrôle du 10 avril au 28 août 1964

Dates	Mâles		Femelles			Total	Moyenne
	T	V	T	N	P		
16.5.64	0	2				2	3,5
7.5.64	0	4	0	1	0	5	
3.6.64	0	0	0	1	0	1	5
4.6.64	1	7	1	0	0	9	
24.6.64	0	4	0	0	1	5	11
25.6.64	4	9	0	2	2	17	
14.7.64	3	9	0	0	1	13	9,6
15.7.64	1	4	0	3	3	11	
16.7.64	0	3	0	1	1	5	
5.8.64	2	5	0	1	2	10	18
6.8.64	3	15	0	3	5	26	
25.8.64	2	10	1	2	1	16	16

b) **Après le deuxième traitement** (deux parties d'HCH pour une partie de distillat de diesel, débit horaire inchangé). Le lendemain du traitement deux glossines ont été capturées sur le bras occidental; l'une d'elles était du groupe Ia (âge de 8 à 11 jours). Sur le bras oriental une femelle du groupe Ia et une du groupe Ib ont été capturées.

La proportion des nullipares augmente: 71% (bras occidental) et 61% (bras oriental).

c) **Après le troisième traitement** (même concentration qu'au deuxième traitement mais débit horaire de 35 litres). Aucune glossine n'a été aperçue sur les deux bras pendant les deux jours suivant le traitement.

Les glossines capturées les jours suivants se répartissent ainsi:

Tableau V. — Effectifs comparés des captures de *G. palpalis gambiensis* Vanderplank sur le bras occidental du marigot Tiao (Haute-Volta) Période de contrôle du 11 avril au 28 août 1964

(Le signe + indique que des glossines ont été aperçues)

Dates	Source					Gué 1					Gué 2					Total	Moyenne
	Mâles		Femelles			Mâles		Femelles			Mâles		Femelles			Toutes catégories	
	T	V	T	N	P	T	V	T	N	P	T	V	T	N	P		
6.5.64		+										+				+	
7.5.64		+										+				+	+
8.5.64		o					o					o				o	
3.6.64		o					+					o				+	
4.6.64		o					o					o				o	0,33
5.6.64		o							1			o				1	
24.6.64		o					+					o				+	
25.6.64		+					+					+				+	+
26.6.64		+					o					o				+	
14.7.64	1					1						+				2	
15.7.64		1					o					o				1	1,25
16.7.64		1					o					o				1	
17.7.64		1					o					o				1	
4.8.64		3					+					+				3	
5.8.64		3	1			1						+				5	3
6.8.64		4	o				o					+				4	
7.8.64		o	o				o					+				+	
25.8.64			+				+					o				+	3,5
27.8.64		3					+				3	1				7	

Bras occidental: 14 mâles ténéraux et 4 vieux mâles à partir du 12e jour,
6 femelles ténérales,
5 femelles nullipares (0–8 jours): 2 le 12e jour, 1 le 13e jour et 2 le 14e jour,
2 femelles pares du groupes Ia (8–11 jours) le 14e jour.
Le % de nullipares atteint 84%.

Bras oriental: 5 mâles ténéraux et un vieux capturé le 8e jour,
3 femelles ténérales,
1 nullipare du groupe Ob (4–8 jours) le 12e jour.
Le pourcentage de nullipares atteint 100%.

Donc aucune glossine femelle capturée n'a atteint un âge suffisant pour déposer sa première larve. Ce troisième traitement a été efficace.

d) **Après le quatrième traitement**

Bras occidental: une glossine a été aperçue le lendemain du traitement. Le 7e jour après le traitement une femelle n'a pu être disséquée en raison de son mauvais état de conservation. Le 13e jour une femelle nullipare (Oa) a été capturée. En outre des glossines ont été vues en 3 occasions.

Bras oriental: une femelle ténérale (Ob) a été capturée le lendemain du traitement et une femelle nullipare (Oa) et à spermathèques vides, le 7e jour. Des

Tableau VI. — Effectifs comparés des captures de *G. palpalis gambiensis* Vanderplank sur le bras oriental du marigot Tiao (Haute-Volta) Période de contrôle du 21 avril au 28 août 1964

(Le signe + indique que des glossines ont été aperçues)

Dates	Gué					2e Point					En amont du pont					Total	Moyenne
	Mâles		Femelles			Mâles		Femelles			Mâles		Femelles			Toutes catégories	
	T	V	T	N	P	T	V	T	N	P	T	V	T	N	P		
6.5.64	1						o					o				1	
7.5.64		o					+					+				+	0,3
8.5.64		o					o					o				o	
3.6.64		o					o					o				o	
4.6.64		o					o					+				+	+
5.6.64		+					o					o				o	
24.6.64		o					+					o				+	
25.6.64		+					o					o				+	+
26.6.64		o					+					o				+	
14.7.64		o					+					o				+	
15.7.64	2					3					1					6	
16.7.64		o				1						o				1	1,75
17.7.64		o					o					o				o	
4.8.64		o				1						+				1	
5.8.64	1						o					o				1	1,25
6.8.64		+				1						o				1	
7.8.64	2						o					o				2	
25.8.64		+						1								1	
27.8.64		+				2		2			1					5	3

glossines ont été vues en 8 occasions. Aucune glossine n'aurait pu déposer sa première larve avant le 5e traitement. (Le cas précédent de la glossine Ob est envisagé dans le paragraphe " Discussion ".)

e) **Après le cinquième traitement** (5e traitement identique au 3e). Aucune glossine n'a été capturée mais des individus ont été aperçus en une occasion sur le bras occidental et en deux occasions sur le bras oriental.

f) **Après le sixième traitement** (identique au 4e). Pendant la période du 27 mars au 10 avril pour le bras oriental et du 26 mars au 10 avril pour le bras occidental aucune glossine n'a été aperçue.

g) **Période de contrôle.** Elle a commencé le 11 avril. Toutes les trois semaines, une enquête a été effectuée pour vérifier les résultats, et la barrière insecticide a été traitée. Le 6 mai, la présence des glossines a été constatée sur les deux bras. A partir de cette date les effectifs de capture n'ont cessé de croître. Les femelles sont rares. Les dernières captures ont été effectuées le 27 août.

V. — DISCUSSION

a) Variation de la population du gîte témoin

On peut penser que la décroissance de la population est un effet des captures pratiquées au même point et qu'après un arrêt de quelques semaines l'effectif

Tableau VII. — Moyenne des effectifs de capture (mâles + femelles) de *G. palpalis gambiensis* Vanderplank pour des périodes de 5 jours (du 17 décembre 1963 au 28 août 1964) sur l'ensemble des gîtes du marigot Tiao (Haute-Volta)

(Entre parenthèses le nombre de jours sur lesquels porte la moyenne)
(Le signe + indique que des glossines ont été aperçues)

Dates	Bras Occidental		Bras Oriental		Témoin
	Moyenne	Observations	Moyenne	Observations	
décembre 15–19	88,5 (2)				
	—				
	—				
janvier	—				
	75,5 (2)				15,5 (2)
1er traitement	—		57* (1)		—
	29,7 (2)	Glossines capturées le lendemain du traitement	—		30 (1)
	28 (1)		8 (3)		17 (1)
février	—		—		—
	50 (1)		24 (1)		—
2e traitement	2 (1)	1 vieux mâle et une femelle Ia le lendemain du traitement	2 (1)	2 pares (Ia, Ib) le lendemain du traitement	48 (1)
	—		—		—
	8 (3)	9 ténérales sur 26 dont 2 femelles (Ia, 1 Oa)	5 (4)	5 ténérales sur 20 (21 février compris) dont 5 femelles nullipares + (1 IIa, 1 IIb)	29 (2)
3e traitement					
	0 (2)		0 (1)		24 (3)
	3,6 (3)	11 ténérales sur 11	3,3 (3)	2 non ténérales sur 10 (femelle Oa, 1 vieux mâle)	22,5 (2)
mars	6,6 (3)	11 non ténérales sur 20 dont 5 nullipares, 2 Ia	0,25(4)	1 Ob (6 mars compris)	10,5 (2)
4e traitement					
	+ (2)		1 (1)	1 Ot	20 (1)
	0,33(3)	1 femelle (?)	0,3 (3)	1 Oa	13 (2)
	0,5 (2)	1 Oa	+ (3)		16,3 (3)
5e traitement					
	0 (2)		0		9 (1)
	+ (3)		+		12 (1)
6e traitement					
avril	0 (3)		0 (4)		14,3 (3)
	0 (3)		0 (3)		7,5 (2)
mai 6, 7, 8 . .	+		0,3	1 vieux mâle	3,5 (2)
juin 3, 4, 5 . .	0,3	1 Oa à spermathèques vides	+		5 (2)
24, 25, 26 . .	+		+		11 (2)
juillet 14, 15, 16, 17 .	1,25	2 mâles ténérales, 3 vieux	1,75	7 vieux mâles	9,6 (3)
août 4, 5, 6, 7 . .	3	11 vieux mâles, 1 femelle ténérale	1,25	5 vieux mâles	18 (2)
25, 27 . .	3,5	6 vieux mâles, 1 femelle ténérale	3	6 vieux mâles, 3 femelles Ot	16 (1)

* Capture non standard l'après-midi

Facing p. 140

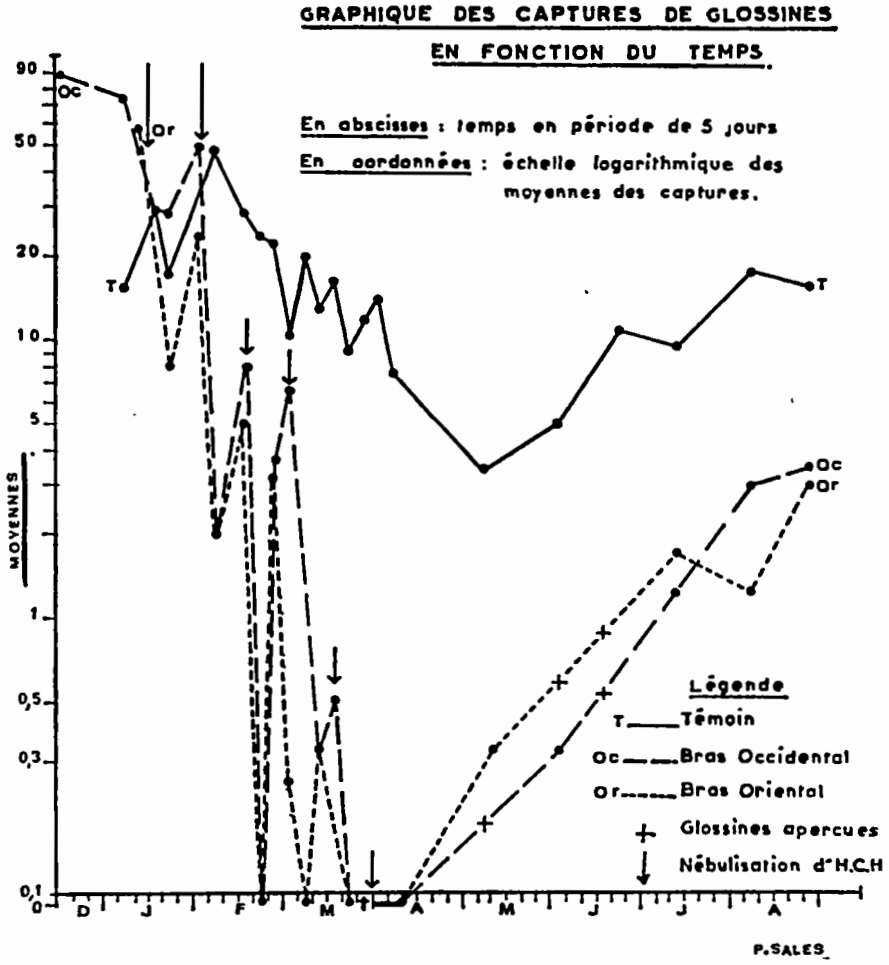

FIG. 2

augmente. On ne peut nier l'effet des captures mais la variation observée correspond à celle que présente habituellement *G. palpalis* (R.-D.) en pays de savane, d'Afrique occidentale, en fin de saison sèche (Nash et Page, 1953).

b) Pourcentage de femelles nullipares

Alors que ce pourcentage a très peu varié pour le gîte témoin, il a considérablement augmenté après chaque traitement pour les deux bras traités.

c) Rémanence du produit nébulisé

Comme la concentration d'HCH et le débit horaire employés au troisième traitement ont provoqué la disparition des glossines pendant deux jours au moins et 5 au plus, nous pouvons admettre que l'HCH nébulisé dans les conditions de l'expérience présente une rémanence de deux jours.

d) **Apparition des glossines entre deux traitements**

L'apparition des glossines entre deux traitements n'est pas rédhibitoire puisque toutes les femelles disséquées ont révélé par leur âge physiologique qu'elles ont éclos après le traitement et qu'elles n'auraient pu déposer leur première larve avant le traitement suivant. Le traitement par nébulisation a donc éliminé la population adulte. Un doute subsiste cependant au sujet d'une glossine qui n'a pu être disséquée et des individus aperçus et non capturés. La glossine ténérale capturée le 7 mars, le lendemain du 4e traitement, pouvait ne pas avoir encore pris une dose létale d'insecticide. Dans le graphique nous n'avons pas tenu compte de cette femelle. Mais le nombre des spécimens observés nous permet de conclure que l'effet de quatre traitements appliqués à intervalles de 15 jours (une partie de distillat de diesel pour deux parties d'HCH, avec un débit horaire de 35 litres) se manifeste par une disparition des glossines pendant quelques jours puis par l'apparition d'individus de plus en plus vieux. Mais les femelles ne peuvent vivre assez longtemps pour déposer leur première larve puisque survient un nouveau traitement.

e) **Réapparition des glossines entre le 1er avril et le 6 mai**

La première femelle capturée le 5 juin était du groupe Oa et a donc éclos entre le 1er et le 5 juin. Buxton (1955) montre dans un graphique la relation entre la température et la durée du stade pupal: à 18° C. la durée du stade est de 73 jours, à 25° C.: 30 jours. Swynnerton (1936) rapporte qu'une *G. swynnertoni* Austen a éclos le 92e jour, dans son milieu naturel.

Le dernier traitement efficace a été appliqué le 4 avril. Donc quelle que soit la durée du stade pupal, cette femelle a été déposée pendant la période des traitements.

f) **Hypothèses pour expliquer la réapparition des glossines après le dernier traitement**

1. — Les glossines ont envahi la galerie traitée à partir des autres marigots soit en traversant la savane par les lignes de crête, soit en traversant la barrière chimique qui aurait été d'une longueur insuffisante ou aurait reçu un traitement non efficace à 100%.

2. — Des glossines ont pu être introduites par l'homme et les animaux.

3. — Le traitement est efficace mais l'intervalle de 15 jours entre les traitements est trop long. Les femelles écloses dès que l'insecticide n'agit plus, peuvent déposer leur première larve. Il faut alors supposer que le cycle ovarien en saison sèche chaude est inférieur à 13 jours (15 jours, moins deux jours de rémanence).

Buxton et Lewis (1934) ont observé que le premier cycle ovarien est compris entre 13 et 24 jours chez *G. tachinoides* Westwood à 30° C. Mellanby (1936) donne le chiffre de 17 jours pour *G. palpalis* (R.-D.).

4. — Les vieilles femelles gravides pourraient être insensibles aux doses expérimentées. Le phénomène de la variation de la sensibilité à différents insecticides (HCH, DDT, Dieldrine) en fonction de l'état physiologique a été observé pour la première fois par Burnett (1962). Nous avons observé le même phénomène (Challier, 1963).

5. — L'insecticide n'a pas été nébulisé en quantité suffisante pour recouvrir toute la végétation du gîte. Or, une bonne couverture insecticide est indispensable puisque le produit n'agit que pendant deux jours au moins (5 au plus). Certains

individus qui se reposent en des points éloignés des bords du lit du marigot, pourraient recevoir une dose trop faible.

g) **Hypothèses mettant en cause les méthodes d'observation**

1. — Les méthodes de capture ne permettent pas de détecter les très faibles densités de glossines. Morris (1961) a montré qu'il faut quelquefois capturer pendant plusieurs jours pour déceler la présence d'une espèce en un point. Il se peut donc que le traitement ait eu une efficacité suffisante pour abaisser la densité de la population au-dessous du niveau où captureurs et glossines ont des chances de se rencontrer

2. — Les groupes d'âge étudiés par Saunders chez *G. morsitans*, au laboratoire, peuvent ne pas correspondre à ceux de *G. palpalis gambiensis* sur le terrain.

h) **Vérification des hypothèses**

Avant de vérifier les hypothèses impliquant l'efficacité de l'insecticide ou la technique de nébulisation il faudrait posséder des données plus précises sur la biologie de *G. palpalis gambiensis*: connaître avec précision la durée du stade pupal dans les conditions naturelles et étudier la relation entre l'âge physiologique des femelles et " l'âge chronologique ". On pourrait alors choisir un intervalle entre les traitements ainsi que la durée de la période que doit couvrir l'ensemble des traitements.

De nouveaux essais pourraient être tentés en choisissant un cours d'eau bien isolé dont une section assez courte serait traitée. Les équipes de captureurs pourraient ainsi parcourir toute la section durant toute la journée pour déceler les faibles densités.

CONCLUSION

Des essais réalisés dans une galerie forestière, en pleine saison sèche de janvier à avril, nous montrent que la nébulisation d'un mélange de deux parties d'HCH pour une partie de distillat de diesel à l'aube et au crépuscle, et à raison de 30 litres de mélange à l'heure (environ 9–11 litres de mélange au km) permet d'abaisser considérablement la population de *G. palpalis gambiensis*.

La rémanence de l'insecticide se situe entre 2 et 5 jours.

Aucune glossine épidémiologiquement dangereuse n'a été capturée entre les traitements appliqués à 15 jours d'intervalle.

Donc, si une nouvelle tactique de lutte contre la maladie du sommeil devait impliquer l'arrêt de la transmission par la double action du dépistage et du traitement de tous les malades d'une part et l'élimination des vecteurs susceptibles d'être infestés d'autre part, deux traitements par nébulisation d'HCH des zones présentant des points de contact homme-glossines pourraient écarter le danger d'une infestation des hommes sains non protégés par les moyens prophylactiques (lomidinisation).

La technique pourrait être appliquée en saison des pluies.

Si le but à atteindre est l'élimination complète du vecteur, il serait nécessaire d'effectuer de nouveaux essais pour préciser certains points tels que la raison pour laquelle les glossines réapparaissent après le dernier traitement. Il serait alors utile d'obtenir dans la nature certaines données biologiques sur les espèces et en particulier:

— quelles sont les limites des groupes d'âge définis par Saunders pour des femelles marquées et lachées dans la nature et recapturées à différentes saisons de l'année;

— quelle est la durée du stade pupal aux différentes saisons de l'année.

RESUME

Pendant la saison sèche 1963-64, des essais de nébulisation d'HCH ont été effectués dans une galerie forestière étroite en zone de savane boisée sur le cours d'eau Tiao, en République de Haute-Volta.

L'insecticide a été employé à raison de 2 parties d'insecticide pour une partie de distillat de diesel. Le produit "Procidacri 100" contient 100 gr. d'isomère gamma par litre.

Pour un débit maximum de l'appareil on a obtenu une consommation d'environ 10 litres de mélange, soit 6 litres d'HCH au kilomètre.

La rémanence du produit se situe entre 2 et 5 jours.

Après chaque traitement les glossines ont disparu pendant au moins deux jours pour réapparaître ensuite, mais toutes les femelles capturées entre les traitements n'auraient pu atteindre un âge suffisant pour déposer leur première larve.

Après deux traitements d'essai, quatre autres ont été appliqués à 15 jours d'intervalle. Aucune glossine n'a été aperçue pendant deux semaines après le dernier traitement.

Diverses hypothèses sont envisagées pour expliquer la présence de glossines constatée un mois après la fin des essais.

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APPLICATIONS PAR VOIE AERIENNE DE TELODRINE DANS LA LUTTE CONTRE *GLOSSINA MORSITANS* WESTW. AU BUGESERA (RWANDA)

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Mission Tsé-tsé au Bugesera

INTRODUCTION

Lorsqu'on décida, il y a quelques années, la mise en valeur du Bugesera, région située dans la partie sud-est du Rwanda, à la frontière du Burundi, on se heurta à un obstacle majeur, la présence de *G. morsitans* et de la trypanosomiase qu'elle transmet. La lutte contre la maladie fut envisagée principalement par l'attaque directe de la tsé-tsé, suivant la méthode de désinsectisation discriminatoire par voie aérienne (" discriminative aerial bush spraying "). Cette méthode de lutte chimique était basée sur celle mise au point par le Tropical Pesticides Research Institute d'Arusha (Burnett *et al.*, 1961) et les données biologiques en ont été exposées dans une courte note (Buyckx, 1963). Une première campagne de désinsectisation fut entreprise de août 1960 à mars 1961 et intéressait une surface de 19.000 ha dont 3.568 ha de biotope furent traités à la Dieldrine (Buyckx, 1961).

Quoique le prix de revient fût compétitif avec celui d'autres méthodes de lutte, on souhaitait que sa réduction soit envisagée. Une étude de la répartition des frais révéla qu'une diminution appréciable de ce prix ne pourrait être obtenue que, d'une part, par la diminution des frais de balisage, de l'autre grâce à une réduction du coût de l'insecticide et une augmentation du rendement de l'avion, ainsi que les chercheurs d'Arusha l'avaient aussi constaté (Burnett *et al.*, 1961). Le rendement de l'avion peut être amélioré par la diminution de la quantité de bouillie à l'hectare et par l'amélioration de la technique du vol de pulvérisation, ce dernier facteur dépendant dans une certaine mesure du balisage. Quant au prix de l'insecticide, l'utilisation d'un produit plus puissant pouvait entraîner une économie appréciable.

La réduction de ces divers facteurs a été tentée lors de la deuxième campagne qui consista dans le traitement, de août 1961 à mars 1962, de 5.321 ha de biotope répartis sur une surface de près de 43.000 ha (Buyckx, 1962). La Télodrine fut essayée parallèlement à la Dieldrine. En effet, dans des essais de laboratoire, Burnett (1961 a) a montré que parmi les hydrocarbures chlorés, seule la Télodrine est supérieure à la Dieldrine; elle est approximativement 2,5 fois plus toxique pour *G. morsitans*, entre autres. Il est arrivé à la conclusion que d'un point de vue pratique, confirmé par les essais en brousse, la Dieldrine est l'insecticide le meilleur et le moins cher et que seule la Télodrine pourrait lui être substituée.

Sur la base des résultats obtenus en 1961–62, il a été jugé intéressant de n'utiliser à l'avenir que la Télodrine et ce fut le cas lorsqu'une troisième campagne fut entreprise en octobre 1963. Elle concernait la désinsectisation de 13.240 ha de biotope sur près de 50.000 ha de " fly belt ".

METHODES ET MATERIEL

Lors de la première campagne, c'est une bouillie à 2,6% de Dieldrine qui fut épandue à la dose de 1,5 l/ha (0,133 gals/acre), soit 3,9 mg/m² de matière active. Pour la plupart des applications, la bouillie fut préparée à partir d'une formulation

spéciale de Dieldrine concentrée à 20% (Dieldrine F1), mélangée dans la proportion de 1 l pour 6,5 l de kérosène lampant (" illuminating kerosene "). L'avion était du type léger, un PA-18 A, équipé d'un moteur Lycoming de 150 hp, d'un réservoir ventral Sorensen en matière plastique de 340 l, d'une pompe aéromotrice et de deux cages rotatives du type " micronair A 100 ".

Le traitement comprenait huit applications à 28 jours d'intervalle et s'effectuait depuis l'aube jusqu'à 8 h 15 et de 17 h au crépuscule, moments de la journée durant lesquels les conditions atmosphériques étaient très favorables à l'épandage d'un aérosol grossier. Le balisage était effectué à l'aide de ballonnets météorologiques de couleur rouge.

Les résultats obtenus furent semblables à ceux de l'essai 1959-60 de Chungai.

Au cours de la deuxième campagne, le même équipement de pulvérisation et la même formulation spéciale furent utilisés, mais d'après les résultats obtenus et l'avis de spécialistes d'Arusha (Burnett et Yeo, communication personnelle), 1 l/ha de bouillie devait suffire pour tuer les glossines et la quantité fut réduite théoriquement à 1,123 l/ha (0,1 gals/acre). En réalité, le débit minimum qu'on put atteindre avec l'installation de pulvérisation équipant l'avion à une vitesse moyenne sol/air de 130 km/h et pour une largeur de bande de pulvérisation de 50 m, correspondait à une dose de 1,107 l/ha. En utilisant une solution à 4% de Dieldrine obtenue par mélange d'une partie de concentré à 20% dans quatre parties de kérosène lampant, on a obtenue un dépôt de matière active un peu supérieur (4,4 mg/m²) à celui de la campagne précédente.

En ce qui concerne la Télodrine, Burnett admet comme dose probable de 14 g/ha (1961 b) à 15 g/ha (1961 a) et Shell recommande 14 g/ha (0,0125 lb/acre). Ces doses ont été établies pour des adultes jeunes des deux sexes. Or Burnett (1961 c) a prouvé que les mouches agées résistaient mieux que les jeunes à l'action de l'insecticide et que les femelles âgées pleines faisaient preuve d'une résistance exceptionnelle. Tenant compte de la probabilité de cette résistance, nous avons épandu 16,6 g/ha, soit 17,8% de plus que la dose recommandée sur la base des essais in vitro. Dans ces conditions, la dose de Télodrine était 2,6 fois moindre que celle de Dieldrine. La préparation commerciale obtenue était de la Télodrine E.C. 15% et une bouillie à 1,5% a été préparée par mélange d'une partie avec une partie de gasoil (diesoline) et huit parties de kérosène lampant. On arrivait ainsi à la même proportion de kérosène que pour la Dieldrine F1.

Les blocs de brousse ont été traités en une seule opération, de façon à éviter des trous dans l'épandage. Le balisage a été effectué à l'aide de balises permanentes érigées dans les blocs, en des endroits favorablement situés pour faciliter au mieux le travail du pilote et atteindre une grande précision dans les passages successifs. On a utilisé des perches d'eucalyptus de 7 à 10 m de long, surmontées par une panneau en unalit ou en tôle ondulée, de 120 × 80 cm, enduit de couleur blanche et sur lequel était peint en noir un chiffre romain. Du fait qu'elles étaient en place en permanence, toutes les balises d'un alignement étaient simultanément visibles pour le pilote qui pouvait, sans risque d'erreur, passer au milieu de deux balises distantes de 100 m. On ne dresse donc qu'une balise pour deux passes et seuls des chiffres impairs I, III, V, VII, IX, XI, XIII et XV figuraient sur les panneaux.

Ce type de balisage présente de sérieux avantages: le signal est visible à plus de 2.000 m et sa permanence rend aisée le repérage du bloc à traiter. D'en haut, le pilote a une vue d'ensemble des passes à effectuer, chaque alignement étant vu en entier, ce qui réduit à peu de chose d'éventuelles erreurs d'écart entre lignes de vol

successives. Mais le principal avantage, c'est que la technique du vol de pulvérisation des vallées étroites a pu être modifié, ce qui augmenta le rendement.

Une autre modification consista dans le raccourcissement de l'intervalle entre applications. Burnett (1961 c) a attiré l'attention sur la nécessité de reconsidérer la conclusion atteinte par Yeo et Simpson (1960), qu'une durée de l'intervalle voisine de celle de la période de pupaison était la meilleure. Au Bugesera, les moyennes mensuelles de la température atteignent 20 à 20,5° C. de février à juillet, 22° d'août à octobre, 21° à 21,5° de novembre à janvier. Ainsi, d'août à octobre, la durée de la pupaison sera d'environ 28 jours et l'âge moyen à l'expulsion de la première larve, 24 jours (Jackson, 1940); de novembre à janvier, respectivement 42 et 25 jours. Donc, à peu près comme dans la partie centrale du Tanganyika (Burnett, 1961 c), les femelles sont pleines et résistantes à l'insecticide à la fin de la troisième semaine après leur éclosion. Cela signifie que pour un intervalle d'application de quatre semaines, toutes les femelles écloses pendant la semaine qui suit la pulvérisation, sont potentiellement résistantes à la suivante, pour des doses cependant mortelles aux jeunes mouches des deux sexes. Un intervalle de 24 jours a donc été choisi à partir de la deuxième campagne.

Pour la troisième campagne, on a disposé d'une formulation spéciale de concentré à 10% de Télodrine dans une huile non volatile, semblable à la Dieldrine F1. La bouillie à 1,5% était préparée par mélange d'une partie de Télodrine 10% à 6 parties de kérosène lampant, correspondant à une dose de 15,8 g/ha.

Le changement du type de cages rotatives " micronair A 1000 " mis à part, méthodes et matériel furent identiques à ceux de la deuxième campagne.

LA REGION DESINSECTISEE

La région du Bugesera et ses conditions écologiques ont été succinctement décrites dans une note préliminaire sur la biologie de *G. morsitans* (Buyckx, 1963). Le Bugesera constitue une zone déprimée, d'altitude moyenne comprise entre 1.320 et 1.500 m, formée de larges plateaux faiblement ondulés, découpés par un réseau dense de vallées sèches ou noyées (lacs et marais). Elle est ceinturée par des collines qui culminent entre 1.600 et 1.800 m. Le biotope de *G. morsitans* se trouve dans la savane arbustive dont la strate supérieure mesure plus de 5 m de hauteur et dépasse rarement 15 m, et dont le couvert ligneux assure un degré de recouvrement très variable, mais ne dépassant généralement pas 30% pour la strate dominante. L'habitat est formé par la partie de cette savane située sur les flancs des vallées et les têtes de vallées colmatées, principalement à partir de mi-pente jusqu'au bas de pente. Les terrains de chasse sont constitués par la savane arbustive d'une hauteur inférieure à 6 m, principalement composée d'*Acacia hockii*, qui se trouve dans l'habitat et/ou le borde. Les acacias recouvrent plus abondamment la zone de colluvionnement au bas des pentes et dans les têtes de vallées colmatées. Les blocs à désinsectiser correspondent donc aux flancs et fonds de vallées.

Essai Télodrine

Le complexe de la vallée Mwesa-Karirisi, situé entre les collines Gako, Muyenzi, Murama, Gatwe, Kabukuba, Katarara, Kalera, Tshyoma et Rwibikara, a été choisi pour l'essai Télodrine car il constitue un ensemble de surface assez importante et est relativement isolé des vallées environnantes. La végétation qui recouvre les collines citées se compose de bosquets xérophiles de densité forte à

moyenne et de lambeaux de forêt sclérophylle, types de végétation impropres au maintient d'une population de *G. morsitans*. Cette vallée et ses embranchements présentent l'aspect typique des vallées sèches à pente transversale faible, à pente longitudinale presque nulle, périodiquement inondées, dont les fonds herbeux à *Themeda triandra* ne portent que quelques buissons et arbustes épars. Au total, la superficie des blocs atteignait 1.119 ha:

Bloc	Mwesa 1	510 ha
,,	,, 2	68
,,	,, 3	52
,,	,, 4	81
,,	,, 5 (Karirisi)	135
,,	,, 6	28
,,	,, 7	10
,,	,, 8	235

Troisième campagne

Pour vérifier l'efficacité des pulvérisations de la troisième campagne, le sentier de capture dit de Kindama fut réouvert un an avant la date prévue pour la première application, le sentier dit de Nyabisindu un mois avant. Ces trajets avaient été tracés en 1957 par l'IRSAC pour l'étude écologique des glossines du Bugesera et les resultats des observations ont été publiés par Van den Berghe et Lambrecht (1962). Ils prouvent qu'une population relativement importante de *G. morsitans* existait dans cette partie du Bugesera. L'ensemble des blocs traités autour du sentier de capture de Kindama dépassait un millier d'hectares. L'autre était également entouré de blocs à désinsectiser.

OBSERVATIONS ENTOMOLOGIQUES

La méthode habituelle des rondes de capture (Buyckx, 1963) a été adoptée pour contrôler l'efficacité de la désinsectisation. Les rondes ont lieu deux fois par semaine pour la capture des glossines, tous les quinze jours pour la récolte des pupes.

Essai Télodrine

En vue de cet essai, un sentier de capture long de 5.000 m, soit 50 piquets distants de 100 m, a été mis en place. Partant de la colline Gako, il descend dans la vallée par le bloc Mwesa 3, la suit en son milieu, puis oblique vers le N–NE pour traverser d'abord la base de la vallée formant le bloc Mwesa 4, puis perpendiculairement la vallée principale (Mwesa 1) et longer ensuite le bas de la pente de l'embranchement ouest (bloc Mwesa 2), et remonter jusque dans la végétation composée de bosquets xérophiles à forte densité. Un sentier allant de Gashora à Myange traverse d'abord la base du bloc 4 en direction N–S, puis obliquant vers l'ouest, la grande vallée Mwesa 1, passe dans le bloc 2 et remonte la pente. A partir du piquet 38 jusqu'au 50, les rondes suivent ce sentier qui connaît un passage régulier de voyageurs et de bétail. La partie traitée commence au piquet 8 et se termine au piquet 45, soit 3.700 m en terrain désinsectisé.

Troisième campagne

Le sentier dit de Kindama commence près du village de Kindama, à la route Kibugabuga–Gihinga qui suit la ligne de crête. Il longe le flanc d'une vallée sèche

qui aboutit au lac Tshohoha Nord, en descendant graduellement pour finir dans le bas de la pente. Il comprend 45 piquets et à partir d'octobre 1962, il a été prolongé de façon à atteindre 5.300 m, soit 53 piquets à l'intervalle de 100 m, aboutissant au milieu du fond de la vallée. A partir du piquet 15, il se trouve dans un bloc désinsectisé.

Le sentier dit de Nyabisindu se trouve au nord de l'extrémité sud-est du lac Tshohoha Sud. Il se compose de 36 piquets, commençant près du lac, remontant la pente, traversant la piste Ngenda–Nemba sur la ligne de crête, pour redescendre dans une tête de vallée sèche aboutissant au lac Tshohoha Nord. Seuls les six derniers piquets se trouvent dans un bloc désinsectisé.

RESULTATS

Essai Télodrine

Vu la date tardive de la décision d'entreprendre la campagne, la Télodrine ne put être commandée à temps. De plus, une erreur de débarquement causa un nouveau retard, de sorte que le nouvel insecticide ne put être appliqué qu'à partir de la cinquième pulvérisation. Les quatre premières le furent donc avec la Dieldrine Fi 20%. Certains blocs n'ayant pu être désinsectisés lors de la première application, une neuvième opération eut lieu, au cours de laquelle un cinquième passage à la Télodrine fut effectué sur le complexe de la Mwesa-Karirisi. Le tableau I donne le résultat des rondes, la figure 1 le nombre de pupes récoltées mensuellement le long du trajet.

Les quatre applications à la Dieldrine, à la dose de 4,4 mg/m², ont été efficaces. La comparaison de ces résultats avec ceux de la première campagne, permet de conclure que non seulement la mortalité des mâles a été élevée, mais également celle des femelles. Ce dernier point est d'ailleurs confirmé par le pourcentage à peine accru de femelles capturées pendant le traitement, par rapport à celui des captures précédant la désinsectisation. Une autre confirmation vient du fait que le nombre de pupes trouvées décroît régulièrement et que déjà après la cinquième application, on n'en trouve plus. Rappelons que la différence entre les deux campagnes réside dans le raccourcissement de l'intervalle entre applications et l'augmentation de la dose par unité de surface.

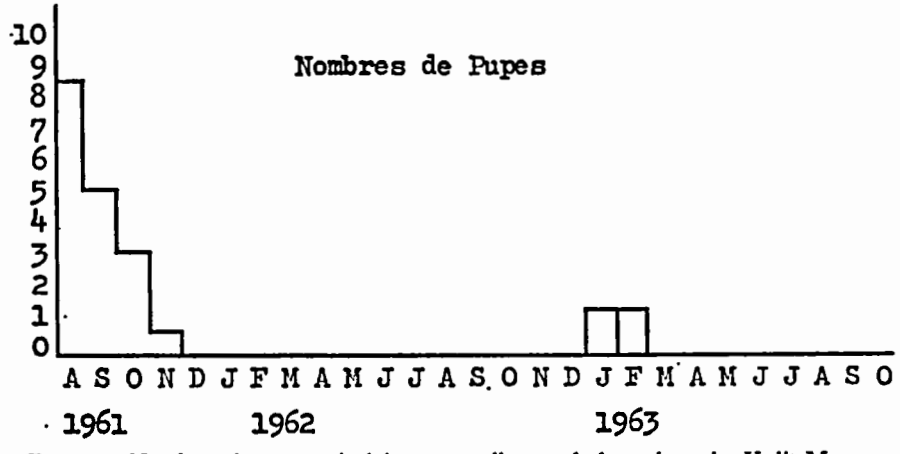

FIG. 1. — Nombres de pupes récoltées mensuellement le long du trajet V dit Mwesa.

Tableau I. — Moyennes hebdomadaires de tsé-tsés capturées le long du trajet V dit Mwesa depuis une semaine avant le traitement jusqu'à la quatrième semaine après la dernière application

Période	Mouches âgées		%	Mouches jeunes	
	Mâles	Femelles		Mâles	Femelles
14.8–20.8 . . .	15,5	5	20,5	0	0
1ère application 22.8					
21.8–27.8 . . .	3,5	0		0	0
28.8– 3.9 . . .	3,5	1	22,2	0	0
4.9–10.9 . . .	2	0		0	0
11.9–17.9 . . .	2,5	0		0	0
2e application 21.9					
18. 9–24.9 . . .	0,5	0		0	0
25. 9– 1.10 . .	1	0		0	0
2.10– 8.10 . .	0,5	0		0	1
9.10–15.10 . .	1,5	0,5	25	0	0
3e application 14.10					
16.10–22.10 . .	0,5	0		0	0
23.10–29.10 . .	0	0		0	0
30.10– 5.11 . .	0	0		0	0
4e application 7.11					
6.11–12.11 . .	0,5	0	0	0	0
13.11–19.11 . .	0	0		0	0
20.11–26.11 . .	0	0		0,5	0
5e application 30.11					
27.11– 3.12 . .	0,5	0	0	0	0
4.12–10.12 . .	1	0	0	0	0
11.12–17.12 . .	0	0		0	0
6e application 21.12					
18.12–24.12 . .	0	0		0,5	0
25.12–31.12 . .	0	0		0	0
1. 1– 7.1 . . .	0	0		0	0
8. 1–14.1 . . .	0	0		0	0
7e application 15.1					
15.1–21.1 . . .	0	0		0	0
22.1–28.1 . . .	–	–		–	–
29.1– 4.2 . . .	0	0		0	0
8e application 7.2					
5.2–11.2 . . .	0	0		0	0
12.2–18.2 . . .	0	0		0	0
19.2–25.2 . . .	0	0		0	0
9e application 3.3					
26.2– 4.3 . . .	0	0		0	0
5.3–11.3 . . .	0,5	0	0	0	0
12.3–18.3 . . .	0	0		0	0
19.3–25.3 . . .	0	0		0	0
26.3– 1.4 . . .	0	0		0	0

Pour obtenir la densité apparente, il suffit de multiplier la moyenne de mâles âgés par 2.

Il est regrettable que la Télodrine ne put être utilisée dès la première application. Le fait que la reproduction semble avoir cessé après la cinquième pulvérisation et les résultats des rondes laissent supposer que la population résiduelle était déjà très faible. D'après les calculs de Yeo et Simpson (1960), le niveau stable de la population résiduelle après quatre applications à 24 jours d'intervalle, doit se situer entre 0,14 et 0,41% de la population originale pour un taux de mortalité de 95% et aux environs de 1% pour 90% de mortalité. D'après les nombres de mâles capturés (31) durant la semaine avant la première application et dans celle immédiatement après (3), le taux de mortalité des mâles a atteint 90%. On peut reprocher à cet essai sa réalisation à partir d'une densité de population relativement faible. En fait, les blocs du complexe Mwesa contenaient le plus de tsé-tsés de tous ceux qui furent désinsectisés au cours de la deuxième campagne. Par contre, le fait que le biotope avait des limites bien marquées et qu'il était bien isolé par une végétation défavorable à *G. morsitans* constituait un avantage.

La capture d'une glossine mâle dans le courant de la deuxième semaine de mars coïncide avec le passage d'un petit troupeau de bétail le long du chemin Mayange–Gashora et il y a tout lieu de croire qu'il provenait de la brousse non désinsectisée.

Les moyennes mensuelles de glossines capturées après l'essai jusqu'en novembre 1963 (tableau II) montrent qu'une lente réinfestation des blocs s'est produite. La moyenne atteint sa valeur la plus élevée en mai 1963, pour retomber à un niveau très bas en décembre. Les faits observés dans d'autres blocs traités uniquement à la Dieldrine montrent également une très lente réinfestation, qui doit être attribuée à l'immigration à partir de la brousse non désinsectisée, certains lambeaux de biotope restant en dehors des blocs traités (Buyckx, 1962). Il paraît opportun de rappeler ici que la désinsectisation a été conçue et menée en fonction du projet de mise en valeur de la région par l'installation d'agriculteurs; elle avait pour but de réduire la population de glossines dans des proportions telles que les risques d'infection du bétail par la trypanosomiase devenaient faibles, et de lever ainsi les obstacles d'ordre psychologique et vétérinaire à l'installation des paysans.

Tableau II. — Moyennes mensuelles de glossines capturées le long du trajet V dit de Mwesa depuis août 1961 jusqu'en novembre 1963

	1961		1962		1963	
	Mâles	Femelles	Mâles	Femelles	Mâles	Femelles
janvier . .			0	0	3,4	0,1
février . .			0	0	1,4	0
mars . .			0,1	0	3,5	0
avril . . .			0,1	0,1	2,3	0,1
mai . . .			0,1	0,1	3,6	0
juin . . .			0,1	0,1	2,2	0,2
juillet . .			0,1	0	1	0,2
août . . .	7,8	1,6	0,2	0	1	0,1
septembre . .	2	0,2	1,4	0,2	2	0,2
octobre . .	0,6	0,4	1,5	0	2,2	0,1
novembre . .	0,1	0,1	0,4	0,1	0,5	0,1
décembre . .	0,3	0,2	1,1	0,1	0	0 (jusqu'au 20.12)

Par souci d'économie indispensable dans des pays aux ressources encore fort limitées, on désirait opérer à un prix de revient à l'hectare libéré aussi bas que possible. L'extinction des tsé-tsés par la désinsectisation n'était pas visée, la disparition progressive de la faible population résiduelle devant être la conséquence du défrichement et du refoulement du gibier. Des facteurs d'ordre politique et économique ont jusqu'à présent retardé l'occupation des terres, causant un décalage de plus en plus important entre cette phase du plan de développement et la désinsectisation.

Quoi qu'il en soit, si on ne peut émettre un avis catégorique sur la valeur de la Télodrine à la dose de 1,65 mg/m² par rapport à celle de la Dieldrine à 4,4 mg/m², on peut toutefois admettre que le traitement paraît avoir eu le même effet que s'il avait été entièrement réalisé avec la Dieldrine.

Troisième campagne

La première application de la troisième campagne était prévue pour début août 1963, afin de cumuler l'action défavorable des conditions climatiques des dernières semaines de la saison sèche et l'effet de la désinsectisation. L'important retard apporté à la livraison du premier lot de Télodrine empêcha l'exécution de ce plan et la campagne ne débuta que le 7 octobre. En règle très générale, les conditions atmosphériques furent très favorables lors de l'épandage et les opérations se déroulèrent de manière très satisfaisante. Malheureusement des événements politiques ont interrompu la campagne au cours de la quatrième application, arrêtant les travaux de la Mission Tsé-tsé pour une durée indéterminée et désorganisant complètement le réseau de rondes de captures. Par conséquent, on ne dispose que des résultats des premières applications.

Des moyennes figurant au tableau III, on peut conclure qu'une population assez importante existait le long du trajet de Kindama avant le traitement, la densité apparente variant de 56 à 126. Les moyennes hebdomadaires de tsé-tsés capturées sont indiquées au tableau IV. Durant les quatre semaines précédant la première application et les trois semaines la suivant, les rondes ont été effectuées à la fréquence hebdomadaire de cinq, afin de pouvoir suivre de plus près la modification du nombre de mouches capturées. Les résultats montrent qu'une mortalité très élevée a été obtenue: dès le quatrième jour après l'épandage, on ne trouve plus de mâles ni de femelles âgées, ce qui laisse supposer un taux de mortalité voisin de 100%. Dans la semaine après la deuxième application, cinq femelles âgées furent récoltées près de la limite du bloc désinsectisé, probablement des glossines immigrantes ou ne se trouvant pas dans le bloc lors de l'épandage. La densité apparente, de 113 avant la première application, tombe à 0 dès qu'elle a été effectuée.

De même pour le trajet dit de Nyabisindu, les captures (tableau V) diminuent brusquement dès la première application pour descendre à zéro en décembre, malgré le fait que 1/6 seulement du trajet se trouve dans la partie désinsectisée.

On peut conclure que l'application de Télodrine avec l'équipement décrit, à la dose de 15,9 g/ha, est très efficace contre *G. morsitans*. Cette dose pourrait être réduite à 14 g/ha.

PRIX DE REVIENT

Par rapport à la première campagne, la réduction de la dose à l'hectare de 1,5 à 1,1 l/ha a permis d'économiser 25% de kérosène; le rendement en hectares par

Tableau V. — Moyennes mensuelles de glossines capturées le long du trajet VIII dit de Nyabisindu depuis août jusqu'à fin 1963

	Mâles	Femelles
août	8,8	0,2
septembre . . .	12,2	0,9
octobre	17,7	1,3
novembre	0,25	0,25
décembre . . .	0	0

Au cours de la deuxième campagne, le prix de revient des opérations de désinsectisation s'est élevé à 354,49 Fr/ha. Il se réparti comme suit: le balisage intervient pour 10,9% le service avion pour 38,9%, l'insecticide pour 47,3% et les frais de transport et le service au terrain d'atterrissage pour 2,9%. Lors des applications de Télodrine, malgré un prix d'achat supérieur de 56%, l'utilisation d'une dose de 16,5 g/ha contre 44,4 g/ha a permis de réaliser une économie de 22,2% sur le coût de l'insecticide. Lors de la troisième campagne, cette réduction a été de l'ordre de 15%.

Au prix de 354,49 Fr/ha, il faut ajouter les frais de mise en place en prenant Bujumbura comme base de départ, soit 25,58 Fr/ha, ainsi que les frais de fonctionnement, soit 9,93 Fr/ha. Le prix de revient s'élève alors à 390 Fr/ha, soit pour une moyenne de 1 hectare traité sur 7,5, 52 Fr/ha.

SOMMAIRE

Dans un essai, la Dieldrine à la dose de 44,4 g/ha a été remplacée pendant la seconde moitié du traitement par la Télodrine à la dose de 16,5 g/ha. Les résultats obtenus indiquent que la Télodrine a une action insecticide sur *G. morsitans* au moins équivalente à celle de la Dieldrine.

Au cours d'un traitement généralisé à une large partie du " fly-belt ", la Télodrine à la dose de 15,9 g/ha a causé un taux de mortalité très élévé et une diminution brutale de la population de mouches âgées.

La substitution de la Télodrine à la Dieldrine a permis de réaliser une économie de 15% sur le coût de l'insecticide, qui intervenait pour 47,3% dans le prix de revient de 354,5 Fr/ha pour la pulvérisation proprement dite au cours de la deuxième campagne. La réduction de la dose à 14 g/ha rendrait l'emploi de la Télodrine économiquement encore plus intéressant.

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Le financement des première et deuxième campagnes de désinsectisation a été assuré grâce à deux subsides mis à la disposition du Service de l'Agriculture du Rwanda par le Vice-gouvernement général du Ruanda-Urundi. Toutes les charges découlant de la direction scientifique et technique, ainsi que la troisième campagne, ont été subventionnées par le Fonds Européen du Développement de l'Outre-Mer de la CEE. Nous remercions vivement toutes les autorités du Rwanda et du Burundi qui ont bien voulu s'intéresser et donner leur appui à ces campagnes de désinsectisation, tout particulièrement M. A. Focan, Directeur régional de l'INEAC R.-U. et M. Cloots, Directeur du Service de l'Agriculture du Ruanda-Urundi.

heure de vol a été augmenté non seulement par la réduction de la dose à l'hectare, mais également par l'amélioration de la technique du vol de pulvérisation. Cette augmentation atteint 23,4%.

Tableau III. — Moyennes mensuelles de glossines capturées le long du trajet VII dit de Kindama depuis septembre 1962 jusqu'en novembre 1963

	1962		1963	
	Mâles	Femelles	Mâles	Femelles
janvier			47,5	11,6
février			42,3	4,9
mars			54,3	7,1
avril			65,5	7,3
mai			68,4	6,7
juin			63,0	5,0
juillet			63,5	3,1
août			57,6	2,1
septembre	31,7	5,5	67,0	1,0
octobre	36,2	4,3	25,2	1,4
novembre	56,1	5,2	3,6	2,2
décembre	60,2	6,4	—	—

Tableau IV. — Moyennes hebdomadaires de tsé-tsés capturées le long du trajet VII dit de Kindama depuis quatre semaines avant le traitement jusqu'à la semaine après la troisième application

Période	Nombre de rondes	Mouches âgées		%	Mouches jeunes		D.A.
		Mâles	Femelles		Mâles	Femelles	
13. 9–19.9	5	56,0	0,4	0,07	2,2	0,4	105
20. 9–26.9	5	70,6	0,6	0,08	2,8	2,0	133
27. 9– 3.10	5	61,0	0,8	0,13	4,6	1,0	115
4.10–10.10	5	59,8	3,4	0,54	0,2	0,6	113
1ère application 10.10 17h.							
11.10–17.10	4	1,7	0	—	1,5	0	3
18.10–24.10	5	0	0	—	2,4	1,4	0
25.10–31.10	5	0	0	—	4,0	3,0	
2e application 1.11							
1.11– 7.11	3	0	1,7	—	1,0	0,3	
8.11–14.11	2	0	0	—	1,5	2,0	
15.11–21.11	2	0	0	—	7,5	3,0	
22.11–23.11	0	—	—		—	—	
3e application 24.11							
24.11–28.11	2	0	0		6,5	1,5	
29.11– 5.12	2	Résultats des rondes perdus après le 21.12 — tsé-tsés rarissimes*					
6.12–12.12	2						
13.12–15.12							
4e application 16.12							
16.12–22.12	2						

* Nous avons eu l'occasion de consulter les formulaires lorsque la quatrième application était en cours.

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THE ERADICATION OF *GLOSSINA MORSITANS MORSITANS* WESTW. IN ANKOLE, WESTERN UGANDA, BY DIELDRIN APPLICATION

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INTRODUCTION

During the first decade of this century the western Tanganyika *G. morsitans* population expanded northwards across the Kagera river, into the kingdom of Ankole, in south-western Uganda. The main advance followed the line of the Orichinga valley and from there spread eastwards through Ishingiro, north-eastwards towards Nyabushozi and north-westwards towards Mbarara. It is believed (Simmons, 1929) that this movement was assisted by the greatly increased human activity in the early years of the 1914–18 War. The cessation of this activity was closely followed by a rinderpest epizootic which decimated both cattle and game, resulting in a marked contraction of the tsetse population towards the Kagera.

By the late 1920's tsetse were again spreading northwards and by the mid-1930's they had reached the Mbarara–Masaka road on a broad front. Attempts to stem this advance by means of sheer cleared barriers failed (Poulton, 1936), and by 1951 it was estimated that some 260 square miles, north of the road, were under fly. In the following year EATTRRO embarked on a scheme to eliminate this salient by means of discriminative bush clearing. This operation (Ford, 1953, 1955, 1956; Harley and Pilson, 1961), while producing a marked reduction in some localities, did not prevent the further expansion of the salient. Thus by 1957 when the experiment was abandoned, at least a further 750 square miles had been occupied and expansion was taking place eastwards into Buganda and northwards towards Toro at an alarming rate.

The Uganda Tsetse Control Department (now the Tsetse Control Division of the Department of Veterinary Services and Animal Industry) then took over, and in order to avert what might well have become a national disaster, it was decided to attempt to halt this advance by shooting out the game animals which form the food supply of *G. morsitans*, a method which had proved to be very successful in other parts of Uganda (Robertson and Bernacca, 1957). Hunting operations were commenced in July 1958 in an area of some 1,200 square miles, later extended to 1,700 because of further advances along the northern and eastern sides of the salient. And, so as to reduce still further the available food supply, all but 5,000 of the 65,000 head of cattle, which had been kept in the area under Antrycide prosalt prophylaxis, were evacuated.

By the middle of the following year, it was apparent that these measures had been justified, the advance eastwards had been halted and tsetse densities generally, markedly reduced. But towards the end of the year, fly succeeded in crossing the Katonga river into Toro, albeit in very light density. A barrier zone, half a mile in depth, was therefore sprayed along the Katonga, using 3% Dieldrin. In all, 35 square miles were treated at a dosage rate of 32 gallons of Dieldrex concentrate per

square mile. This measure was completely successful and no further catches were recorded on the Toro side of the river. Later, an additional 11 square miles were treated to prevent a possible break-out of fly through the extreme north-east corner of the operations area. There, however, because of the denser vegetation rather more insecticide was used—55 gallons of concentrate per square mile. Spraying was completed by August 1960, and was again completely successful.

As hunting continued, the progressive disappearance of their food supply led to the actual elimination of tsetse from more and more of the operations area. Thus by November 1962 it was possible to withdraw all the hunters from western Buganda. Hunting was, however, continued in Nyabushozi, Ankole, but by September 1963, hunting in the northern part of the country had been completed and what few hunters remained were stationed on the southern periphery to prevent the ingress of game.

The use of game elimination as a means of eradicating tsetse from northern Ankole had, however, provoked vociferous opposition from wild life conservationists, and it was therefore decided to eradicate fly from the remaining 600 square miles of country still occupied, north of the Mbarara–Masaka road, by means of insecticide. Assistance for this project was sought from sources outside Uganda, and eventually the United States, through their Agency for International Development, contributed insecticide, vehicles, tentage and radio equipment.

During 1962 considerable preparation by way of track making and camp building was carried out in the area to be reclaimed and spraying was actually commenced on 25 February 1963.

DESCRIPTION OF THE AREA

That part of eastern Ankole in which the spraying operation was carried out is moderately hilly country lying at an elevation of between 4,000–4,700 feet. Across the south-west corner there is, however, a range of rather more prominent hills, of which Kishasha (5,612 feet) is the highest. Many of the open valleys which intersect the area support seasonal swamps, but only in the north-western corner are there seasonal streams, tributaries of the Rusangwe.

The mean annual rainfall here is 35–40 inches, except in the north-west, which is rather wetter, receiving 40–50 inches. April–May and September–November are the periods when rainfall is heaviest. There are two dry seasons, of which the most pronounced is that in June–July; the other, in December–February, is less severe. The mean annual minimum temperature lies between 12·5 and 15° C. and the maximum between 25 and 27·5° C.

Because of regular burning, the vegetation is an Acacia fire climax savannah woodland, in which the dominant trees are *Acacia gerrardii* and *A. hockii*, with *A. sieberiana*, *A. vermoesenii* and *Albizzia coriaria* also present. Ant-hill thickets composed of such species as *Grewia mollis*, *G. similis*, *Allophyllus africanus*, *Rhus natalensis*, *Capparis erythrocarpos* and often dominated by *Euphorbia candelabrum*, are numerous in valleys. The characteristic grass of the area is *Themeda triandra*, it has however been replaced in several localities by *Cymbopogon afronardus*. Other common grasses are *Loudetia kagerensis*, *Brachiaria spp.*, *Setaria spp.* and *Hyparrhenia spp.* Towards the southern edge of the area is a sizeable tract of grass savannah known as **Rwanda–Orwera** in which the dominant species are *Hyparrhenia dissoluta* and *Themeda triandra.*

A varied, but no longer abundant, game population inhabits the area, of which the characteristic species are Buffalo, Eland, Zebra, Waterbuck, Topi and Impala. Reedbuck, Bushbuck, Duiker, Oribi, Warthog and Bushpig are ubiquitous. Roan Antelope and Klipspringer do occur, but are distinctly rare. Of the carnivors, Lion, Leopard, Spotted Hyena, Jackal and Hunting Dogs are present. There are no elephant resident in the area, but it is occasionally visited by herds from the Katonga.

EARLIER INSECTICIDAL WORK

The first attempt to eradicate *G. morsitans* in Ankole by insecticidal means was a joint exercise with the Colonial Pesticides Research Unit, carried out in the Kabiganda valley, a tributary of the Kagera, between October 1957 and September 1958.

There, the putative resting sites on the undersides of branches of *Acacia gerrardii*, up to a height of 10 feet in areas of double-storey vegetation were sprayed with a single application of 3% Dieldrin emulsion Eclipse knapsack sprayers fitted with extension lances and special nozzles. Apart from one block in which thickets were also treated, only an average of 6·75 trees per acre were sprayed, over an area of some 11,000 acres, 1,300 gallons of Dieldrex concentrate were used, i.e. 75·52 gallons per square mile. Estimations showed that a deposit of 0·8 gm. of Dieldrin per square foot had been laid down.

Complete eradication was not achieved, the tsetse population was, however, considerably reduced.

In the expectation that the further eradication of tsetse in Ankole from areas beyond that being reclaimed by game elimination, would have to be by insecticidal means, a spraying trial was carried out in a 5 square miles block of Acacia savannah woodland at Rushozi in central Nyabushozi county in August and September 1959. There, all trees over 4 inches in diameter were sprayed to a height of 13 feet—larger trees on three sides, smaller trees on two sides. A 3% Dieldrin emulsion, prepared from Dieldrex 15T, was applied by means of Leo-Colibri knapsack sprayers, at a rate of 42·7 gallons of concentrate per square mile. The sprayed block was surrounded on all sides by fairly heavy fly, yet the interior of the block remained fly free from early November until June of the following year.

A further trial to determine the effect of bush fires on Dieldrin deposits and also the feasibility of using a more dilute emulsion was carried out at Mizi, in Nyabushozi, in July–August 1961, using the same techniques as were employed at Rushozi. Two blocks were sprayed with a 3% emulsion, one of which was burnt before being sprayed and the other after, while a third block was treated with a 2·2% emulsion after being burnt. The rate of application for the three blocks was 70·2 gallons, 69·16 gallons and 53·26 gallons of concentrate per square mile respectively. Only in the block sprayed with 3% emulsion, after being burnt, were satisfactory results obtained.

In January 1962 a more comprehensive trial was carried out in the Nyankumba valley in Nyabushozi, as it was then expected that an early start would be made on reclaiming the area remaining under fly north of the Mbarara–Masaka road.

The purpose of this trial was to test the efficiency of the proposed organisation and technique, which had been evolved from experience gained in earlier trials and during operational spraying along the northern periphery of the Ankole hunting area

and in Bunyoro. A further purpose of this trial was to determine the effect of spraying on mammals and birds, in response to the apprehension expressed by wild life conservationists.

2,713·8 acres were sprayed, using a 3% Dieldrin emulsion, at a dosage rate of 50·2 gallons of 18% concentrate per square mile. The output per sprayer/day was 14·3 acres using 6·7 gallons of 3%. On the last two days of this trial, working under very favourable conditions an average output of 19·1 acres per sprayer/day using 8·25 gallons of 3% was achieved.

As far as could be ascertained the spraying did not have a deleterious effect on the mammalian and avian fauna, the tsetse population was, however, markedly reduced. Complete eradication was not however achieved, presumably because the treated block was surrounded on all sides by tsetse populations from which immigration must have occurred.

THE SPRAYING OPERATION

Organisation

Tracks and Camp Unit

Because of the size of the area to be reclaimed, over 600 square miles, in which prior to the operation there were very few means of access for motor vehicles, a tracks and camps unit was formed which constructed several hundred miles of motorable track, thus dividing the area into some eighty-six blocks, varying in size between 2 and 10 square miles, their shape and size being governed by the terrain. This unit also erected base camps at Nyakashashara, Nsikizi, Rwebigemano, Muko, Kyenshama and Akayanja for the use of the spraying units—temporary forward camps were, however, built by individual spraying units as and when required.

Observation Unit

In the area reclaimed by hunting it had been found that as the tsetse population declined, a point was reached at which they could not be caught by routine patrols but could still be caught by intensive surveys using bait cattle, but during the final stages of the disappearance of fly, although they were no longer being caught by the tsetse catchers accompanying the herds, individual animals in these herds were still becoming infected with trypanosomiasis. It was therefore believed that if a test herd could be kept in a particular locality for a minimum period of six months without reinfection, then that area could be regarded as being tsetse-free.

An observation unit was therefore set up to supervise the introduction of test herds and the carrying out of the supplementary surveys and other observations. As blocks were sprayed, test herds each of ten head of cattle were progressively introduced, every herd being required to graze over the whole of the area within 3 miles of its boma. Eventually, forty-eight herds were introduced, their distribution was however limited by the availability of water. A survey team was therefore employed to carry out intensive surveys in those localities which could not be examined with test herds. The cattle for these herds had all been obtained from tsetse-free country, and prior to their introduction they were treated with a sanative dose of Berenil. After introduction all the animals in each herd were blood-slided at fortnightly intervals, and the slides examined for trypanosomes. In the event of an animal being found to be infected, the whole herd was treated with Berenil.

Spraying Units

The actual spraying was carried out by spray teams consisting of ten spray operators equipped with knapsack sprayers with ten porters to carry insecticide. A spray team supervisor was in direct control of the operators, while a ganger organised a continuous supply of insecticide. During the course of spraying, individual machines inevitably broke down from time to time, and each team was therefore provided with a mechanic to carry out, as far as possible, repairs on the spot. Accompanying each mechanic was a reserve operator, carrying a spare machine, whose function was to take the place of anyone dropping out for repairs, thus ensuring the smooth progress of the operation. The demarcation of the area covered by each team was the responsibility of the slasherman who cut blazes on trees with a panga along the edge of the sprayed zone. A tsetse assistant was in overall charge of each team, and these were grouped together in threes to form spraying units, commanded by tsetse officers. Two such units were employed in the reclamation scheme proper, while a third was raised to speed up the spraying of a zone overlapping the area being hunted. In addition to the spray teams, each unit also had an insecticidal mixer, who prepared the 3% emulsion, a storeman, a nightwatchman, two camp maintenance porters and a runner, as well as the drivers and van boys of the unit's vehicles, which consisted of a light vehicle (a " Scout ") for an officer-in-charge, three 3-ton lorries to transport spray teams and their equipment, and a 500-gallon tanker lorry to provide water for mixing insecticide, washing machines and protective clothing, and domestic consumption.

Headquarters

A base camp was set up at Sanga, adjacent to the main Mbarara–Masaka road, from where the entire scheme was controlled by the senior officer-in-charge. Here the accounting, both financial and stores, was carried out; there was also a spraying machine repair section—which carried out more detailed repairs and overhauls than could be effected by mechanics in the field—and a vehicle repair section.

MATERIALS

Insecticide

A 3% wt./vol. emulsion of Dieldrin, prepared from Shell Chemical Company's Dieldrex 15T (18% Dieldrin wt./vol.) emulsible concentrate, diluted with water.

Spraying Equipment

Leo-Colibri knapsack spraying machines, fitted with " 700 " lances having ceramic nozzle discs with an aperture of $\frac{3}{64}$ inch. The output is controlled by means of a Cooper-Pegler pressure regulating valve, set to deliver at a constant pressure of 20 lb. per square inch with the spray-gun trigger fully depressed, at which pressure both adequate throw and coverage can be obtained.

TECHNIQUE

In preparation for spraying, machines were pumped to a pressure of 45 lb. per square inch with air, then to 80 lb. per square inch with insecticide, each machine being loaded with 1·6 gallons (7·274 litres).

As from the results of the Kabiganda trial, it is considered that a deposit of

80 mgm. of Dieldrin per square foot should be laid down, and that with the equipment described above, the rate of discharge is 1·6 gallons in ten minutes; thus presuming that an average swathe width is 9 inches, the rate of coverage has to be as near as possible to six linear feet per second. Sprayer operators were therefore trained to spray at that speed.

From observations on the resting sites of *G. morsitans* in Ankole, it was considered that they were insufficiently sharply defined to permit highly selective spraying, especially as there is no marked seasonal contraction of range, and as this was not an experiment, but a reclamation operation, 100% success was imperative. The trunks of all trees, other than *Acacia hockii* under 6 feet high, were therefore sprayed, from grass level to a height of 12 feet, including the undersides of branches up to this height. Small trees were sprayed on one side only, larger trees on two sides and Euphorbias and other large trees on three sides. Thickets were sprayed with a broad cone and given up to three swathes, depending on their size. It was expected that under favourable conditions each sprayer operator would be able to cover 20 acres per day using 10 gallons of 3% emulsion.

PROCEDURE

Spray teams paraded at 0730 hours for charging spraying machines with air, these and 150 gallons of diluted insecticide in 5-gallon drums, which had been prepared the previous evening, were then loaded on to the team's lorry, the team then embarked and at 0800 hours proceeded to the site of that day's spraying. On arrival at the site the equipment was unloaded and the spraying machines were charged with insecticide. The sprayer operators, each accompanied by a porter carrying a 5-gallon drum of insecticide and a charge pump, then formed up on the base line at 25-yard intervals—this interval was dependent on the density of the bush and was narrower in dense woodland, but wider in, for example, tree savannah. The first sprayer operator stationed himself at 12½ yards from the blaze marking the edge of the previous day's spraying. The slasherman was stationed at the other end of the line, some 12½ yards beyond the last sprayer operator. Because it was found that too long a line of sprayer operators was very difficult to control, the second spray team allowed the first a short interval of time to get ahead before it moved off, so that eventually the three teams moved forwards through their area in echelon formation. The use of compasses as a means of controlling the direction of march was abandoned after the Nyankumba trials as it was found their use did not improve the efficiency of the operation, as any good slasherman could produce a sufficiently straight line for the purpose of the exercise.

During the actual spraying, one half of each team was supervised by the spray team supervisor and the other half by the tsetse assistant, their particular functions being to check the spacing of the sprayer operators, their spraying speed and the care with which the insecticide was applied, thus ensuring adequate coverage.

After the first recharging of machines, some of the porters decanted their insecticide into the drums of other porters in order to fill them and they then returned to the transport for a further supply, an operation which was carried out progressively as the day's work proceeded.

The distance covered by a spraying team each day was governed by the shape of the block and the density of the vegetation, nevertheless approximately 2½ miles were covered each day.

On the completion of the spraying, the team returned to camp where they thoroughly cleaned their machines in a special area—the cleaning compound constructed for the purpose—after which they moved to the washing compound and washed themselves, free soap being provided.

Insecticide for the following day was prepared each evening in the cleaning compound—adjacent to which the insecticide store was sited. The drums used for the mixing and carriage of insecticide were also stacked in this compound when not in use.

Unless the work had been interrupted by such factors as inclement weather or transport breakdowns, spraying was only carried out on five days each week, Saturday morning being devoted to overhauling equipment and vehicles and washing protective clothing.

In order to prevent, as far as possible, the burning of blocks which had already been sprayed, some days were devoted, whenever weather conditions were favourable, to the burning of blocks ahead of the spraying front.

PROGRESS

The first stage of the operation was the spraying of a zone along the eastern half of the periphery of the area to be reclaimed by spraying, overlapping that being reclaimed by hunting. Having completed this, spraying then continued through the eastern half of the reclamation area, on a broad front, covering the whole of the country to as far west as the open grasslands of Rwanda Orwera. Meanwhile, in June, a third spraying unit had been raised to continue westwards the spraying of the zone overlapping the hunting area.

As the main operation approached the Mbarara–Masaka road, work was commenced on the sheer clearing of a barrier zone, along the northern side of this road, in order to isolate the sprayed area from the tsetse populations to the south. This clearing, which was done on contract by the Mowlem Construction Company, using tractors and chains was commenced at the Ankole-Buganda border and from there was progressively extended westwards, reaching Kishasha hill in January 1964, by when 84 square miles had been cleared. Near Sanga the existence of a sizeable tsetse population adjacent to the road on the southern side necessitated the extension of this clearing across the road.

By December the spraying of the eastern half of the operations area had been completed, after which work on the western half was commenced, beginning in the zone overlapping the hunting area, from where the operation was progressively extended southwards. The spraying of this overlap zone was completed in February—Unit No. 3 had meanwhile been transferred to Bunyoro. The amount of spraying which had to be done in this western region was reduced by some 8,000 acres as a result by mechanical bush clearing at Rihengire for the ranching development scheme there.

Spraying continued smoothly through the first half of 1964, but in July both units had to be withdrawn and sent to the eastern half of the area, to Mizi, where a tsetse had been caught and the cattle in the test herd there had become infected with trypanosomiasis. A block of just over 20 square miles was resprayed, covering the area grazed by the infected herd and its environs. The spraying units then returned to complete the western half, Unit No. 2 finished their assignment in August and Unit No. 1 theirs in early September.

By the time the scheme was completed, an estimated total of 644·21 square miles had been sprayed, using 25,218 gallons of 18% Dieldrin concentrate, i.e. a dosage rate of 39·1 gallons per square mile. As 18,707 sprayer/days had been required, the average output per sprayer/day was therefore 22 acres, using 8·1 gallons of 3% emulsion. Only a 79·8% efficiency was achieved during the operation, as out of a total of 23,435 potential sprayer/days only 18,707 were actually used for spraying. Bad weather caused the greatest loss—1,266 sprayer/days, closely followed by moving to and building forward camps—1,200. Motor vehicle breakdowns accounted for 730, administration 482, sickness and absenteeism 338, repairing tracks damaged by rain 300, grass burning 200, spraying machine breakdowns 172 and demonstrations 40.

ENTOMOLOGICAL RESULTS

As far as can be determined, this spraying operation has been completely successful, only one *G. morsitans* has been caught within the area sprayed, at Mizi, after which that block was resprayed. The introduction of test herds began in September 1963 since when a total of forty-eight herds have been progressively distributed. All the animals in these herds are blood-slided at fortnightly intervals and to date only seven animals have been found to be infected with trypanosomiasis, three of them at Mizi, before that block was resprayed.

The danger of fly being carried into the reclaimed area from south of the Mbarara–Masaka road is fully appreciated and seven pickets have been set up adjacent to the road to de-fly pedestrians and vehicles moving northwards along tracks into this area.

Work on the next stage of the total eradication of *G. morsitans* in Ankole has, however, already commenced with the spraying of a zone 2 miles in depth along the southern side of the Mbarara–Masaka road, which is shortly to be followed by the mechanical clearing of some 70,000 acres of tsetse habitat. These measures will materially reduce the risk of fly reoccupying the area which has been reclaimed.

COSTS

The total expenditure incurred by the Uganda Government up to the end of September 1964 on this scheme amounted to £157,566, made up as follows:

	£
(1) Salaries of officers	15,758
(2) Wages of staff	69,881
(3) Allowances, subsistence, leave, etc.	4,299
(4) Operation of vehicles	18,179
(5) Stores, sprayer spares, uniforms, insecticide	5,932
(6) Spraying equipment	1,997
(7) Prefabricated houses	2,643
(8) Vehicles	2,706
(9) Mechanical clearing	36,171

In addition, the United States A.I.D. contributed 23,200 gallons of Dieldrin, three personnel-carrying lorries, two tanker lorries, six light vehicles (" Scouts "), vehicle spares, tents and radios, to the value of £59,420, thus bringing the total cost of the scheme to £216,986.

The expenditure by the Uganda Government covers not only the actual spraying scheme and the associated observational work, but also the cost of the preliminary trials, training exercises and other preparatory work.

If the cost of all the capital equipment is disregarded, and the cost per acre so calculated only from the recurrent expenditure, i.e. items (1) to (5) of the Uganda contribution—£114,049, plus the cost of the insecticide provided by AID—£36,550, a total of £150,599, then the cost per acre of the 412,307 acres sprayed amounts to 7/30, i.e. £233/12sh. per square mile.

Such costs do not, however, give a true picture of the efficiency achieved; owing to the delays in the arrival of the vehicles etc. required, the preliminary stages were considerably prolonged and therefore the expenditure on them, disproportionate. Further, no allowance has been made in the above calculation for the depreciation of capital equipment which, presuming an average life of five years, should amount to approximately $\frac{3}{5}$ of its value, i.e. £26,455, thus bringing the total cost to £177,054, i.e. £275/16sh. per square mile or 8/58 per acre.

A more realistic estimate of the cost per acre can be obtained from a detailed study of the costings and output of Units No. 1 and 2, during the time they were actually engaged in spraying. The average monthly cost of operating one of these units amounted to Shs. 17,767/- made up as follows:

	Shillings
Operation of vehicles	4,180/-
Wages	11,933/-
Allowances	732/-
Stores, protective clothing, sprayer spares, etc. .	922/-

The average monthly output was 16·66 square miles at a dosage rate of 40·1 gallons per square mile, and as the cost of Dieldrin used was Shs. 32/- per gallon, the expenditure per square mile on insecticide amounted to Shs. 1,283/20, while the labour and other costs amounted to Shs. 1,068/80—a total of Shs. 23 52/-, i.e. £117/12sh. or 3/68 per acre.

DISCUSSION

Apart from economies which would have been achieved had the preliminary stages of this operation not been so prolonged, only the adoption of a more selective spraying technique would have enabled the cost to be further reduced. As already mentioned, however, there is in Ankole no marked seasonal contraction of range by *G. morsitans* and this has been especially true during the period of the operation, when the abnormally wet conditions which have prevailed in Uganda since 1961 have meant that the dry seasons have not been so severe nor so prolonged. More than one visitor to the scheme expressed the view that fewer trees per acre could possibly be sprayed; such suggestions were however disregarded as this was a reclamation exercise not an experiment and a margin for error, the size of which could not de determined, had to be allowed. As the cost of respraying might well be even higher than the initial application, because of the disturbance of routine involved, spraying rather more trees than is perhaps necessary during the original spraying is therefore justified, if by doing so a 100% kill is obtained and the risk of having to respray avoided.

A comparison of the costs of this operation with those of other insecticidal schemes is, with the exception of the Kabiganda experiment (Hocking, 1961), not

perhaps strictly valid, because of the different conditions under which they were carried out. The cost of the Kabiganda work was £250 per square mile, using a rate of application of 75·7 gallons of 18% Dieldrin per square mile, costing Shs. 52/50 per gallon. Since that experiment was carried out labour costs in Uganda have more than doubled, but the price of Dieldrin has fallen, that used in this present scheme cost only Shs. 32/- per gallon.

The work of Kirkby and Blasdale (1960) in Northern Nigeria, using DDT in the dry season habitat of *G. morsitans*, cost £98 per square mile or £116 if the capital depreciation on vehicles and equipment is included. It would, however, appear that both this spraying and that reported by Davies and Blasdale (1960) in which the costs, not counting capital depreciation, were reduced to £86 per square mile, was very much more selective than the present scheme.

SUMMARY

Between February 1963 and September 1964, 644·21 square miles of Acacia savannah woodland in eastern Ankole were sprayed with a 3% emulsion of Dieldrin, at an average dosage rate of 39·1 gallons per square mile, in order to eradicate *G. morsitans*.

The total costs of operation, including preliminary trials, spraying and observational work, amount to £275/16sh. per square mile.

As far as can de determined to date the operation has been completely successful.

Acknowledgements

The success of this operation is entirely due to the careful planning of Mr. J. P. Bernacca and Mr. M. A. Gimson, and the devotion to duty of Mr. K. H. F. Scott, Mr. E. Mulindwa, Mr. S. Sorensen, Mr. D. Kiberu, Mr. P. Masolo, Mr. J. Bingworuguru and their staff.

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AN ATTEMPT TO ERADICATE *GLOSSINA TACHINOIDES* WESTW. AND *GLOSSINA MORSITANS SUBMORSITANS* NEWST. IN AN AREA OF ENDEMIC SLEEPING SICKNESS IN NORTHERN NIGERIA BY THE USE OF RESIDUAL INSECTICIDES

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Ningi Chiefdom, the area under consideration, is located about 55 miles NNW of Bauchi in Northern Nigeria and its principal features are shown in Fig. 1. Ecologically this is a transition area between the Sudan savannah zone and the Northern Guinea savannah zone of Nigeria (Keay, 1953).

Surveys conducted in this area since 1953 have revealed two active foci of sleeping sickness. Originally it was hoped to achieve control of the disease by treatment of the cases discovered in these surveys. However, new cases continued to be found at an active focus 5 miles to the south-west of Ningi town. Two factors contributed to the failure of this purely therapeutic approach; firstly in any population there may always be an unknown reservoir of *Trypanosoma gambiense* (Richet, 1964) which the present field diagnostic technique of neck lymph gland examination fails to discover in a single survey of the population; the second factor is associated with the daily movement of people and the practical difficulty of tracing everyone from a particular village area for examination.

It was therefore decided to combine drug treatment with control of the vector, *G. tachinoides*, in an attempt to ensure that the transmission cycle of the disease was broken.

CONTROL MEASURES UNDERTAKEN

As it was known that *Glossina* spp. retreated to the riverine vegetation in the dry season (Nash, 1937), it was hoped that the control measures undertaken to eradicate *G. tachinoides* would simultaneously eradicate *G. morsitans submorsitans*, the vector of bovine trypanosomiasis.

All riverine vegetation to a height of 5 feet was sprayed with a suspension of 5% DDT, prepared from 75% wettable powder. To isolate the area from fly reinvasion, a 4% aqueous solution of Dieldrin, prepared from an emulsion concentrate containing a minimum of 20% Dieldrin, was applied to the riverine vegetation at the periphery of the DDT sprayed zone.

In all, a total amount of 10,176 lb. of DDT was applied to 328 miles of river, giving a rate of application of 31 lb. per mile; 230 gallons of Dieldrin emulsion concentrate were applied to 55 miles of river, giving a rate of application of 4·1 gallons per mile.

For continued isolation of the control area, it was proposed that the Dieldrin barriers should be resprayed at the start of each dry season.

RESULTS

To determine the success of the insecticidal measures, thirty-six standing fly catch points were established to check whether fly had been eradicated and if the Dieldrin barriers were preventing reinvasion.

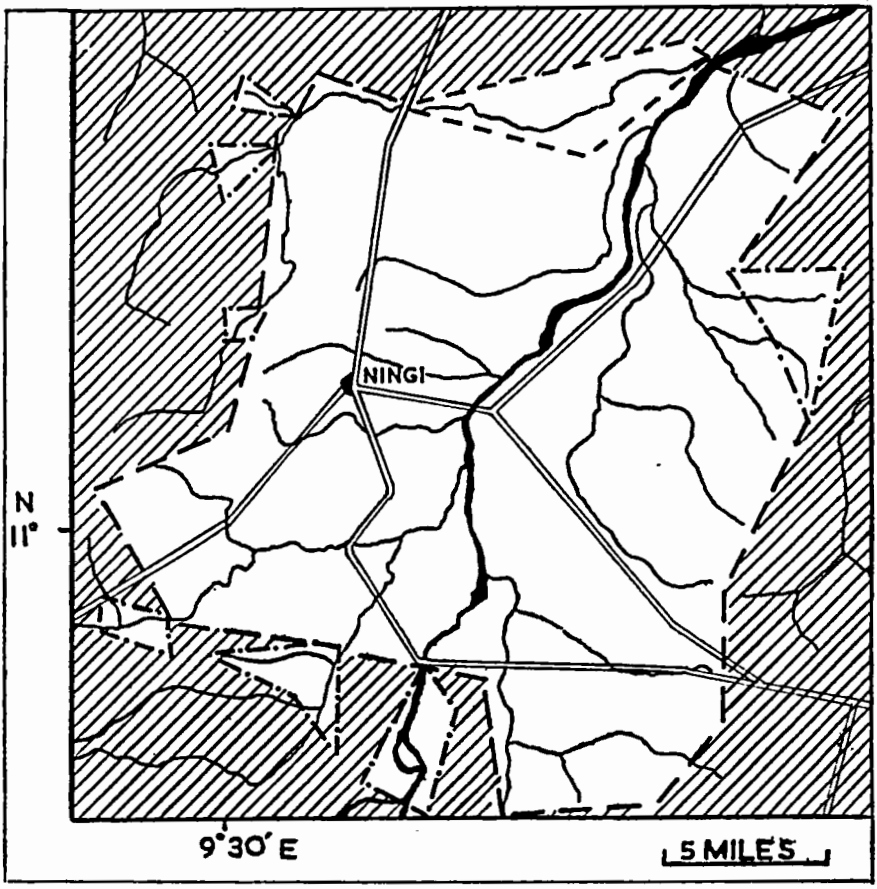

FIG. 1.—Map of Ningi chiefdom to show details of eradication area.
—— —— All rivers inside sprayed with 5% DDT.
— . — . — All rivers inside sprayed with 4% Dieldrin—i.e. the Dieldrin barriers.
— — — — All rivers inside have had vegetation cleared as a localised control measure in the past.
//////// Area in which no control measures undertaken.
═══ Roads.

Up to the end of October 1964, 6½ months after the completion of the control programme (10 January to 12 April), two *G. tachinoides* had been caught at the same point in the centre of the eradication area. It is considered that an isolated pocket of fly managed to survive in an area missed by the spraying. It is proposed to spray out this pocket at the start of the 1964–65 dry season.

COSTS

A total of £1,568 4s. 0d. was spent on insecticide, £1,088 3s. 0d. on DDT and £480 1s. 0d. on Dieldrin, giving a cost of £3 4s. 7d. per mile for DDT and £7 16s. 0d. per mile for Dieldrin. Labour costs amounted to £1,064 12s. 7d.; transport to £159 17s. 11d. This gives a grand total of £2,792 14s. 6d., excluding the salaries of permanent staff engaged on the scheme.

DISCUSSION

To achieve success in breaking the transmission cycle of human sleeping sickness there must be complete eradication of the vector, so that the potential source of infection in a population, the *T. gambiense* reservoir, cannot give rise to further cases.

Originally it was proposed to isolate the eradication area from reinvasion with cleared barriers; however, the wages for labour to clear these barriers would have made the total cost of the eradication scheme impracticable. The decision to use Dieldrin for barrier purposes was based on the fact that, in past eradication schemes undertaken by the Sleeping Sickness Service, Dieldrin emulsion concentrate had been found to have a longer residual life than DDT wettable powder.

It is assumed that the attempt to eradicate tsetse flies with DDT has met with success and that Dieldrin used as a barrier is effective in keeping the sprayed area free from reinvasion by *Glossina* spp. from nearby foci outside the eradication zone.

SUMMARY

A brief outline is given of a sleeping sickness control scheme undertaken in Northern Nigeria, in which a combination of chemotherapy and vector control by residual insecticides was used in an attempt to break the cycle of transmission.

It is considered that the method of vector control adopted was successful, as Dieldrin barriers effectively prevented reinvasion of the DDT-sprayed zone by tsetse flies from adjacent infected areas.

Acknowledgements

The advice and encouragement of Dr. M. P. Hutchinson, Sleeping Sickness Advisor, Ministry of Health, is gratefully acknowledged, as is valuable criticism of the manuscript by Mr. D. A. T. Baldry, Senior Research Officer, Entomology Section, West African Institute for Trypanosomiasis Research. This paper is published with the approval of the Permanent Secretary, Ministry of Health, Northern Nigeria.

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SECTION III

HUMAN TRYPANOSOMIASIS/TRYPANOSOMIASE HUMAINE

LA TRYPANOSOMIASE HUMAINE RESIDUELLE A *TRYPANOSOMA GAMBIENSE* EN AFRIQUE FRANCOPHONE DE L'OUEST

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I. BILAN ACTUEL DE L'ENDEMIE TRYPANIQUE AU SEIN DE L'OCCGE

Lors de la dernière Réunion du CSIRT (Conakry, juillet 1962), nous avons eu l'honneur d'exposer l'état de l'endémie trypanique dans les huit Etats africains[1] membres de l'OCCGE et, plus brièvement, en quelques autres Etats francophones ou anglophones non-membres de notre Organisation (voir Rapport final de la Réunion de Conakry — CCTA Publication No. 88, pages 283 à 300, huit graphiques).

Depuis, la situation déjà si satisfaisante comparativement à celle des années 1940 n'a cessé de s'améliorer régulièrement, progressivement au sein des Etats africains membres de l'OCCGE touchés, si sévèrement jadis, par la trypanosomiase (Côte d'Ivoire, Dahomey, Guinée, Haute-Volta, Mali, Sénégal).

Le graphique d'ensemble de la page 174 traduit bien cette amélioration en montrant que le nombre des nouveaux trypanosomés dépistés a été de:

2.923 en 1961
3.209 en 1962
2.855 en 1963 (pour 8.222.891 habitants visités)

mais que l'index de contamination nouvelle[2] (incidence pour l'OMS) qui correspond pratiquement à l'index de virus en circulation, tombe de:

0,06% en 1960 et en 1961
à: 0,049% en 1962
et 0,034% en 1963

Aucun nouveau foyer important de trypanosomiase n'a été découvert en 1962 et en 1963.

En 1963, l'activité prospective d'ensemble a battu tous les records, encore qu'elle ait été bien ralentie en deux Etats par des circonstances indépendantes des Services sanitaires.

Les index de présence aux prospections ont été satisfaisants dans l'ensemble, signant une aide administrative de qualité non négligeable, sauf en certains rares cas locaux.

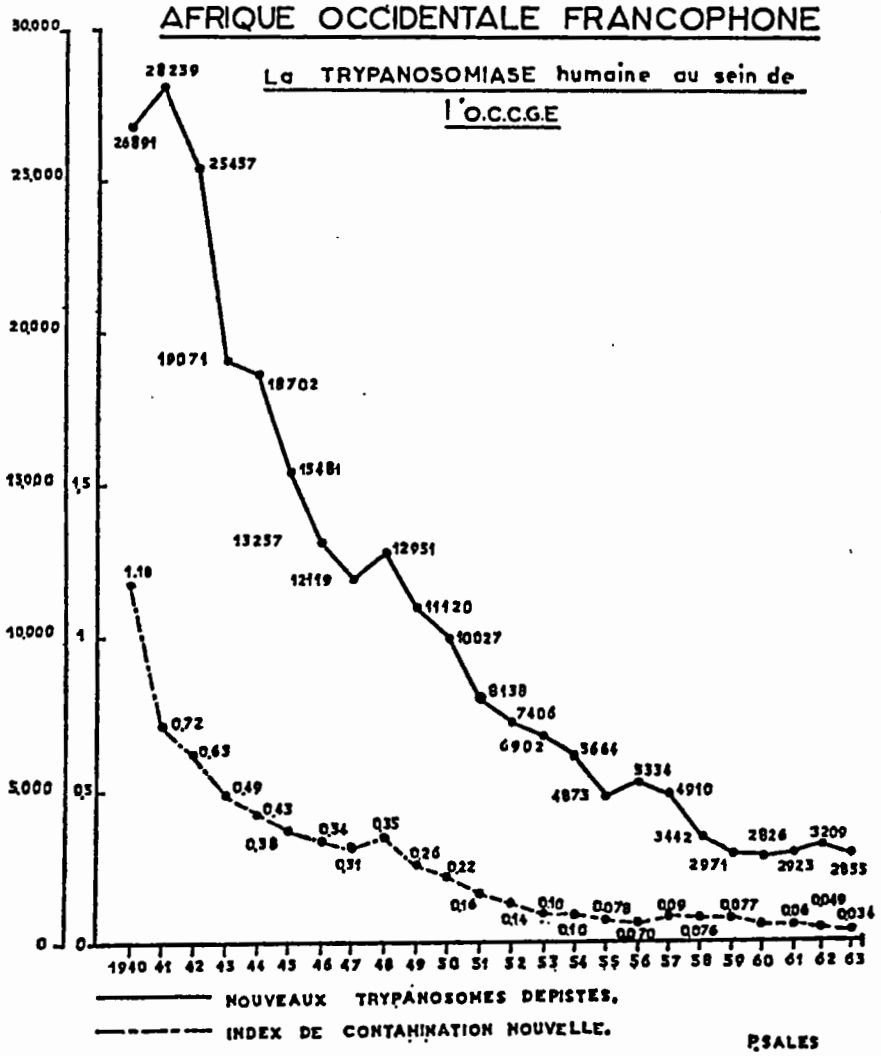
Il se confirme que le renforcement constant de l'infrastructure " Secteurs " en plusieurs Etats (Haute-Volta, Côte d'Ivoire) tendant vers l'idéal d'un Secteur normal pour pas plus de 300.000 habitants, le perfectionnement continu des méthodes de dépistage et de traitement autorisent l'espoir raisonné d'un amenuisement progressif du réservoir " trypanosomes " au cours des années à venir, sous

[1] Côte d'Ivoire, Dahomey, Guinée, Haute-Volta, Mali, Mauritanie, Niger, Sénégal. Le Togo a manifesté son désir d'adhérer à l'OCCGE en fin 1964.
[2] Pourcentage des nouveaux trypanosomés dépistés dans l'année par rapport aux habitants visités.

réserve impérative que les efforts et moyens actuels et les indispensables crédits de fonctionnement soient intégralement maintenus, voire encore renforcés.

Une action conjointe de nos Etats membres avec certains Etats anglophones frontières (Nigéria surtout Nord[3] et Est et Ghana) apparaît de plus en plus indispensable pour approcher encore plus vite l'éradication de la trypanosomiase.

Le manque persistant de renseignements et de liaisons en ce qui concerne le Ghana pourtant si important pour nos Etats frontières à tant d'égards — trypanosomiase, lèpre, tuberculose, onchocercose, méningites, variole, rougeole, etc. — est une fois de plus bien vivement regretté.



[3] 1.478 nouveaux trypanosomés dépistés en 1963 dont 1.443 pour le seul Nord-Nigéria — 20 millions d'habitants environ. Nous tenons à remercier ici bien vivement Monsieur le Ministre de la Santé Publique du Nigéria pour les états statistiques hebdomadaires et mensuels complets qu'il nous a fait tenir avec tant de régularité et d'amabilité.

En ce qui concerne la fameuse prospection conjointe, entre Guinée — Sierre-Leone — Libéria, du pays Kissi, ce problème étudié en mi-1962 par d'éminents experts de l'OMS — Professeurs Vaucel et Waddy — fut mis à l'ordre du jour de la 9e Réunion du CSIRT tenue à Conakry en août 1962. En septembre 1963, une réunion d'experts tenue au Siège de la CCTA à Lagos et à laquelle furent invités à participer des techniciens de l'OCCGE (Médecin Général Inspecteur Richet; Directeur du Centre Muraz; M. Challier, entomologiste, détaché de l'ORSTOM; Directeur du Service des Grandes Endémies de la Côte d'Ivoire) décida la mise sur pied d'une mission préliminaire, financée par l'AID des U.S.A., devant œuvrer trois mois en pays Kissi à compter de la fin du premier trimestre 1964 et qui serait composée de deux épidémiologistes (un francophone, un anglophone), d'un entomologiste et d'un vétérinaire.

Une Commission restreinte étudia ce problème dans le cadre de notre dernière Conférence ministérielle inter-Etats de Conakry en novembre 1963. Malheureusement, l'absence à la dite réunion de représentants du Libéria, de la CCTA et de l'AID ne permit pas de propositions fermes à l'époque. Toutefois, depuis fin 1963, les contacts étroits que nous avons pris et maintenus avec le Secrétariat Général de la CCTA ont permis de mettre sur pied cette mission préliminaire, financée en partie par l'AID des U.S.A. et qui, réunie à Conakry le 2 avril 1964, a la composition suivante:

Epidémiologistes

— Docteur Hutchinson, du Ministère de la Santé Publique du Nigéria Nord: Chef de Mission.
— Docteur Barry Abdoulaye, Inspecteur de Secteur de N'Zérékoré contrôlant actuellement la région Kissi de la Guinée: Epidémiologiste francophone.

Entomologiste[4]

— M. Brengues, de l'ORSTOM, détaché à l'OCCGE, pour étude des glossines du foyer Kissi et des mesures à proposer contre le vecteur.

Vétérinaire

— Devait être procuré par l'IBAH. A été finalement fourni par la Guinée.

Cette mission préliminaire en cours et qui aura une durée probable de trois mois permettra de délimiter exactement:

— l'action conjointe à entreprendre dès la saison sèche de fin 1964,
— les moyens à mettre en œuvre: prospection, traitement, prophylaxie, campagnes antiglossines pour enfin venir rapidement à bout du vieux foyer Kissi. Si nécessaire, lors de la vraie campagne conjointe qui doit logiquement commencer en saison sèche, vers fin 1964, l'OCCGE pourra mettre à la disposition des trois Etats une petite équipe et un camion-laboratoire pour recherche des hyper-bêta-2-macroglobulinémies, d'où perfectionnement des méthodes de détection en ce vieux foyer Kissien si intéressant à bien des égards.

[4] De plus l'OCCGE participe encore à cette mission préliminaire par l'envoi pendant quelque temps, en Guinée, de M. Challier, entomologiste de l'ORSTOM, détaché à l'OCCGE, accompagné de captureurs.

II. AMELIORATION DES TECHNIQUES DE DEPISTAGE

A. Recherche des suspects à hyper-bêta-2-macroglobulinémies (en abréviation: HB_2M)

Lors de la neuvième Réunion du CSIRT (Conakry, août 1962) nos camarades de l'OCCGE, Bentz, Carrie, Ducasse, Macario et Perier ont insisté (Rapport final de cette 9e Réunion, pages 387 à 391) sur le très grand intérêt de la recherche systématique de la B_2M sérique en prospection.

A la Conférence technique de Bobo-Dioulasso de mars 1963 (P.V. final, tome 2, pp. 326-344) le Pharmacien-Capitaine Bentz et le Médecin-Capitaine Carrie ont clairement exposé ce qui peut être espéré de la recherche de l'augmentation du taux des B_2M dans le sérum aux points de vue **diagnostic** et de ses fluctuations en fonction du traitement (**pronostic** et **contrôle** rationnel du traitement).

En Haute-Volta, ces faits ont reçu confirmation au cours du 2e semestre 1963 dans le vieux foyer de Niegho et Nabou à l'occasion d'une petite reviviscence détectée en mars 1963.

Fin avril 1963, 1.633 habitants firent l'objet d'une enquête sérologique qui révéla une HB_2M dans 57 cas, à savoir:

— 7 anciens malades traités (sur 14 revus)
— 4 chez 4 NT +
— 46 chez des villageois non trouvés T + et qui furent systématiquement traités à la Lomidine ou à l'Arsobal selon l'état de leur L.C.R.

Un contrôle sérologique en juillet révéla que 3 seulement de ces 46 suspects n'avaient pas modifié leur B_2M d'une façon significative.

En janvier 1964, une nouvelle prospection-contrôle fut opérée: aucun nouveau NT ne fut dépisté.

48 des HB_2M d'avril furent examinés sérologiquement
11 seulement présentaient encore une légère HB_2M
24 porteurs de ganglions eurent aussi un examen sérologique:
2 se révélèrent HB_2M et furent traités.

Les conclusions du Médecin Lieutenant Colonel Labusquière, Directeur du Service des Grandes Endémies de la Haute-Volta, sont les suivantes:

1) Dans tout foyer de reviviscence, une recherche systématique des HB_2M doit être entreprise.
2) Tous les HB_2M, même non-porteurs de trypanosomes visibles, doivent être traités, de préférence à l'Arsobal.
3) Le reste de la population doit être lomidinisé.
4) Grâce à cela, n'importe quel I.C.N. peut être ramené à zéro.

Tout le monde ne sera peut-être pas d'accord sur la proposition n° 2) et certains souhaiteront sans doute encore que les suspects à HB_2M fassent l'objet d'examens répétés, exhaustifs, variés (triples centrifugations, moëlle sternale, inoculations aux animaux réceptifs, etc. . . .), en somme soient " passés au peigne fin " ne serait-ce que pour avoir enfin la preuve de ces trypanosomiases latentes, " incipiens ", de ces porteurs apparemment sains qui, bien mieux que les hypothétiques réservoirs animaux domestiques ou sauvages de *T. gambiense*, expliqueraient la persistance de certains foyers dits résiduels, voire de ces guérisons spontanées que nous n'avons personnellement encore jamais vues.

Il semble que nous approchions désormais de très près la solution de ces passionnants problèmes, grâce à la recherche des HB_2M. Aussi bien, l'étude en cours d'un tout petit foyer voltaïque, celui de Douroula (Secteur de Dédougou), va-t-elle sans doute nous permettre d'enrichir encore nos connaissances:

Avec 3 NT+ en 1962 et 3 NT+ encore en 1963, Douroula fait à l'époque actuelle figure de petit foyer et a provoqué une enquête sérologique portant sur lui et deux autres petits villages voisins: Sa et Souma.

Sur 1.336 prises de sang, 33 HB_2M ont permis la découverte de 2 NT+.

Les HB_2M de Sa et de Souma ont été systématiquement traités:

— au Mel.W.-Lomidine pour ceux qui sont onchocerquiens,
— à l'Arsobal pour les autres.

Les HB_2M de Douroula sont simplement soumis à une surveillance étroite.

Tout le reste de la population a été lomidinisé.

De nouveaux contrôles vont être effectués.

Au Sénégal, les vieux foyers historiques de la Petite Côte (canton de la Somone), des Niayes et du Niombato sont en voie d'amenuisement.

En fin 1963, le Directeur du Service national des Grandes Endémies du Sénégal nous a signalé que, dans le village de Kopgoyam-Diogoye (Petite Côte), sur 734 visités, 7 ganglionnaires avaient été reconnus porteurs de trypanosomes. Les autres furent lomidinisés. Le Médecin Commandant Mattern, de l'Institut Pasteur de Dakar, constata que 34 habitants présentaient un taux augmenté de B_2M et deux d'entre eux furent encore trouvés porteurs de trypanosomes.

La méthode de la recherche systématique des HB_2M a donc permis de déceler 2 NT de plus et a, par ailleurs, montré que les 7 premiers NT + avaient eux aussi une augmentation marquée de leur B_2M.

Le Médecin Colonel Lacan et le Médecin Commandant Mattern ont décidé de rechercher l'HB_2M chez les 25.000 à 30.000 habitants de ce vieux foyer de la Petite Côte dans l'espoir de parvenir enfin à son éradication. Le docteur Mattern qui a mis au point une technique spéciale compte pouvoir faire 500 tests par jour, ce qui permettrait de terminer les examens à la fin du premier semestre 1964.

Les deux médecins attirent l'attention une fois de plus sur:

— l'intérêt très grand de la recherche de l'HB_2M chez tous les habitants des vieux foyers de trypanosomiase;
— la nécessité de multiplier les examens, de " fouiller à fond " le diagnostic chez des HB_2M éminemment suspects et qui permettront parfois de trouver 3 à 4 fois plus de NT+ que la prospection normale la plus minutieuse;
— la nécessité de ne pas lomidiniser prophylactiquement avant d'avoir effectué ces examens puisque l'on sait que les diamidines peuvent " masquer " le trypanosome et rendre impossible la mise en évidence du flagellé.

En Côte d'Ivoire, en janvier 1964, le Ministre de la Santé Publique de la Côte d'Ivoire a bien voulu demander l'aide de l'OCCGE pour réduire le foyer résiduel Abengourou et zone rurale périphérique sur un rayon de vingt kilomètres (canton Indénié surtout) où 267 NT+ ont été dépistés en 1963, soit près de 60% de l'ensemble du Secteur Adzopé-Dimbokro-Bondoukou, de loin le plus touché des 15 Secteurs de la Côte d'Ivoire.

Depuis 1955, date de la création du Secteur, 300 à 700 NT+ sont dépistés chaque année dans le foyer d'Abengourou-Agnibilékrou.

La demande d'aide de la Côte d'Ivoire ayant été acceptée d'enthousiasme par le Secrétaire Général de l'OCCGE et le Centre Muraz a fait l'objet des mesures suivantes:

— Enquête entomologique de fin février à mi-mars sous la direction de MM. Challier et Eyraud, de l'ORSTOM, détachés à l'OCCGE.
— Envoi le 23 mars 1964, à Abengourou, du Pharmacien-Capitaine Bideau, chef de la Section Chimie-Immunologie (Centre Muraz) de l'OCCGE, chargé d'y organiser une enquête par électrophorèse sur les B_2M parmi les habitants du foyer grâce à un des camions laboratoires de la Côte d'Ivoire spécialement équipé à cet effet.
— Formation pour ces recherches spéciales au Centre Muraz en avril du médecin-chef du Secteur d'Adzopé et de personnels infirmiers.

Il serait bien séduisant d'utiliser la méthode lors de la future prospection conjointe du vieux foyer Kissi en fin 1964 — début 1965.

B. Recherche des trypanosomes dans le sang

Il semble décidément que cette recherche ne soit pas " payante " sous certaines latitudes: Haute-Volta, pour 1963, 16.741 examens de sang n'ont permis de " raccrocher " que 4 NT+ qui eussent échappé au dépistage sans le placard de Ross. Ce n'est tout de même pas négligeable.

Des constatations identiques sont faites depuis vingt ans dans les autres Etats membres de l'OCCGE.

Et pourtant, l'on continue de parler des fameuses formes sanguines pures dans le foyer Kissi en Sierra Leone et au Libéria.

La " prospection conjointe " de = 1965 nous renseignera sans doute sur ce point si intéressant.

C. Rôle de l'AMA

Nous ne nous lasserons jamais de répéter que le personnel d'AMA a un rôle de plus en plus important à jouer à mesure que la trypanosomiase semble s'effacer progressivement de la scène tragique qu'elle occupa durant des lustres. C'est dans les consultations de dispensaire qu'on trouve maintenant le plus de trypanosomés, et en période **avancée** parmi les fébricitants, algiques, petits psychiques venant réclamer des soins.

Les dernières Réunions d'Experts (OMS, Genève, juin 1962; CSIRT, Conakry, août 1962) ont particulièrement insisté sur ce point—cf. P.V. final de notre Conférence de Nouakchott, octobre 1962, tome 2, pp. 31, 35, 145.

Malheureusement, les personnels d'AMA connaissent de moins en moins la trypanosomiase, n'y pensent pratiquement jamais, ne la recherchent pas systématiquement. Le passé est oublié.

La palpation ganglionnaire systématique, la goutte épaisse à tout fébricitant ne sont plus des gestes automatiques. Aussi serait-il bon que tout personnel d'AMA lise au moins une fois les émouvants rapports du grand Jamot que l'on retrouve encore dans les archives. Peut-être leur lecture inciterait-elle certains praticiens à faire un meilleur examen tout à la fois de leurs malheureux patients et de . . . leurs propres compétence ou conscience professionnelles.

D. Chimio-prophylaxie

Rappelons qu'en Afrique francophone de l'Ouest elle n'est appliquée qu'une

fois par an aux villages présentant un I.C.N. d'au moins 1 % ou (Haute-Volta) à ceux présentant un trypanosomé notoirement autochtone.

Le tableau ci-dessous indique cette activité par Etats-membres courant 1962 et 1963.

Etats	Nombre de Lomidinisations		Observations
	1962	1963	
Côte d'Ivoire . . .	66.101	50.294	
Dahomey . . .	0	0	
Haute-Volta . . .	7.557	6.116	
Guinée	2.157	3.948	
Mali	12.018	17.893	
Sénégal . . .	18.905	10.162	
Totaux . . .	106.738	88.413	

Nous sommes évidemment bien loin des chiffres records — 55.383 en 1953 — des années héroïques (1947-57) de la lomidinisation intensive au sein de l'ex-Service Général d'Hygiène Mobile et de Prophylaxie de l'Afrique Occidentale Francophone.

Cette diminution est bien normale et elle est fonction de la régression constante du nombre des foyers ou de leur importance.

Comme au cours des années précédentes, aucun incident n'a été signalé courant 1963. Aucun diabète post-diamidinisation n'a été porté à notre connaissance depuis le début des lomidinisations dans l'ex-A.O.F. et dans l'ex-A.E.F.

E. Thérapeutique

Le rapport annuel 1963 de la Haute-Volta comporte une partie " thérapeutique " très intéressante.

L'observation des fiches de 325 NT+ et de 78 " anciens " montre que:

82,6 % des " 1ère période " ont été traités à l'Arsobal **sans incident** (protocole n° 56-bis à 3 injections)

et 78,4 % des " 2ème période " l'ont été avec 3 décès (1,5 % d'accidents mortels).

Au Secteur 7, le Médecin Capitaine Carrie poursuit l'expérimentation du Mel.W. et du Mel.W.-Lomidine.

Mel.W.

Le docteur Carrie porte un jugement qui semble assez sévère sur ce produit qu'il accuse de 5 décès **tardifs** — de cause inconnue ou diverse — parmi les 65 malades en " 2ème période " traités.

Parmi les 60 autres:

1 rechute, 1 échec, donc 58 bons résultats

7 malades en " 1ère période " ont été traités et blanchis sans accident ni échec

L'injection unique de Trimélarsan semble donc l'idéal pour les malades en " 1ère période ".

Pour les " 2ème période " il semble que l'on doive s'orienter vers un schéma type " Neujean-Arsobal ", selon l'atteinte du L.C.R., avec une dose de 5 mg/kg.

Mel.W.-Lomidine

Le 11.953 RP ne semble pas constituer une très bonne thérapeutique et le Professeur Schneider préconise, d'ailleurs, de ne l'utiliser que pour la chimio-prophylaxie. Le docteur Carrie lui attribue cependant une action eutrophique et préparante chez des malades en mauvais état qui ne supporteraient pas d'autre thérapeutique — Arsobal notamment (cf. P.V. final, Conférence Conakry, pp. 423-438).

Dans son excellent rapport annuel 1963, le Médecin Lieutenant Colonel Labusquière a essayé de dresser le bilan des différentes thérapeutiques appliquées en Haute-Volta en se basant sur les résultats obtenus chez 319 NT+ traités en 1963 et sur 3306 NT traités les années précédentes.

Il résume cette étude sous forme de tableaux:

1ère Période

Resultats	Lomidine	Arsobal Protocole O	Arsobal Protocole 56-bis
Décès	0	0	0
Retrouvés T+	0,7%	1,9%	0
Passés en 2ème période	6,4%	3,0%	1,1%

Pour les malades en 1ère période, le traitement par l'Arsobal 56-bis ne semble donc pas se discuter. **Il faudra beaucoup de mérite au Mel.W. pour faire mieux.**

2ème Période

Resultats	Lomidine Tryparsamide	Arsobal 56-bis ou	Arsobal " Neujean "	Moranyl Tryparsamide
Décès par trypano ou thérapeutique	2,5%	1,6%	0,3%	4,6%
Retrouvés T+	0,7%	0,8%	1,1%	0
Echecs	9,6%	5,7%	5,5%	18,6%

Ou encore

Resultats	Anciens traitements	Arsobal
Décès par trypano ou thérapeutique	3,1%	1,4%
Retrouvés T+	0,5%	0,8%
Echecs	12,4%	5,6%

La cause semble donc, à tous points de vue, entendue en faveur de l'emploi de l'Arsobal.

Il est possible de calculer d'autres pourcentages, par exemple:

Ceux des guéris + EOST + disparus après contrôle normal

On obtient pour les " 1ère période ":

avec la Lomidine 67,9%
avec l'Arsobal protocole O 81,1%
avec l'Arsobal 56-bis[5] 77,7%

[5] Ce traitement, étant plus récent, offre moins de recul pour classer les malades parmi les guéris. Le chiffre de 77,7% est donc certainement " pessimiste " par rapport à la vérité.

et pour les " 2ème période ":

avec la Lomidine-Tryparsamide	56,5%
Arsobal 56-bis ou 58-bis	71,0%
Arsobal " Neujean "	62,6%
Moranyl-Tryparsamide	51,0%

Ceux des décédés de cause intercurrente ou inconnue

1ère Période

Lomidine	3,7%
Arsobal protocole O	1,3%
Arsobal 56-bis	0

2ème Période

Lomidine-Tryparsamide	4,4%
Arsobal 56-bis ou 58-bis	1,9%
Arsobal " Neujean "	2,6%
Moranyl-Tryparsamide	8,4%

Comme conclut le Médecin Lieutenant Colonel Labusquière:

" Quel que soit le bout par lequel on prenne la question, il s'avère qu'il n'y a aucune comparaison possible entre les anciens traitements classiques et l'Arsobal, tout plaidant en faveur de ce dernier qui devra être employé chaque fois qu'il n'y aura pas de contre-indication formelle en attendant qu'une décision officielle soit prise pour le Mel.W."

En conclusion, pour les NT, l'idéal semble:

— 1ère période: Arsobal protocole 56-bis à 3 injections
— 2ème période: Arsobal protocole " Neujean "

Evidemment, la question est plus délicate pour les **anciens échecs** repris en traitement, pour lesquels le docteur Labusquière a pu toutefois établir le bilan suivant:

Traitement	Nombre de traités	Décès par trypanosomiase	Décès par cause intercurrente ou inconnue	Total des guéris + EOST + disparus après contrôle normal
Moranyl-Trypar.	76	7,4%	11,9%	38,1%
Lomidine-Trypar.	29	3,4%		51,7%
Arsobal 56-bis ou 58-bis	73	3,9%	1,3%	42,1%
Arsobal " Neujean "	55	5,4%		65,4
RECAPITULATION				
Anciens traitements	105	5,2%	6,9%	40,0%
Arsobal	128	4,6%	0,7%	53,1

Là encore l'Arsobal remporte la palme et, singulièrement, le protocole de notre ami Neujean, ce qui se comprend aisément puisqu'il s'agit d'échecs repris en traitement.

Ces différents tableaux nous montrent aussi combien les fameux décès dits par " cause inconnue ou intercurrente " doivent souvent être dus, en fait, à la trypanosomiase — directement ou parfois plus ou moins indirectement. Nous le soupçonnions déjà et nous remercions le remarquable Directeur du Service National des Grandes Endémies de la Haute-Volta et ses meilleurs médecins-chefs de Secteurs de nous en apporter une preuve qui nous semble difficilement discutable.

Ce bilan thérapeutique, ardu certes, est extrêmement méritoire et scientifiquement satisfaisant et répond au vœu que nous avions formulé dans nos Instructions n° 2350/END/TRY du 30 avril 1958 (ex S.G.H.M.P. de l'A.O. francophone).

A Bamako les premiers résultats publiés sur l'expérimentation au Mel.W. après un recul de 2 ou 3 ans ont paru très favorables (P.V. de la Conférence interministérielle de l'OCCGE tenue à Conakry en novembre 1963, pp. 439-442). Nous souhaitons que le docteur Sow puisse nous les confirmer au cours de nos prochaines Conférences de l'OCCGE.

Les quelques décès signalés " par affection intercurrente " nous inquiètent toujours quelque peu et l'on peut se demander s'ils ne s'apparentent pas aux décès dans les mois suivant la cure signalés par le docteur Carrie.

Nous pensons aussi à l'article du Professeur Collomb[6] portant, il est vrai, sur des trypanosomés atteints de troubles psychiques qui font sans doute de ces malades un terrain particulièrement sensibilisé.

Il semble bien que le Mel.W. soit une excellente thérapeutique de la T.H. à *T. gambiense* en 1ère période mais que nous devions encore prudemment réserver notre opinion sur son innocuité et son efficacité comparées à celle des produits mélaminyl (Arsobal) en ce qui concerne la "2ème période".

Rappelons qu'il semble peu efficace et dangereux dans la T.H.A. à *T. rhodesiense* (Aphed, Robertson, Willett) contre laquelle le Mel.B. agit beaucoup mieux.

CONCLUSION

La régression progressive de l'endémie trypanique au sein des six Etats africains membres de l'OCCGE ou elle sévit encore — la Mauritanie a toujours été indemne et la trypanosomiase semble avoir disparu du Niger depuis plusieurs années — permet d'espérer aboutir dans un avenir proche à un amenuisement frisant l'éradication.

A condition, bien sûr, que soient intégralement maintenus, voire encore renforcés, l'infrastructure " Secteurs des Services nationaux des grandes Endémies des Etats " et tous les moyens de fonctionnement des dits Secteurs[7].

Mais la puissance sans cesse accrue de nos méthodes de prospection et de diagnostic — notamment, recherche systématique des HB_2M et, prochainement, sans doute techniques de l'immuno-fluorescence — de nos moyens de traitement, la poursuite de la chimio-prophylaxie diamidinique dans les rares foyers subsistant encore, la mise en œuvre ou la poursuite de campagnes insecticides anti-glossines dans ces mêmes foyers — Mali, Côte d'Ivoire, Haute-Volta — autorisent vraiment l'espoir raisonné d'amenuiser d'année en année le réservoir de *T. gambiense* dans ses derniers foyers résiduels.

[6] Collomb, H., Zwingelstein, J., Ayats, H., et Seck, L. Les incidents et accidents de la trypanosomiase par le Mel.W. A propos de 38 observations. *Bull. de la Société Médicale d'Afrique Noire de langue française.*

[7] 81 Secteurs sont actuellement en action au sein des huit Etats africains membres de l'OCCGE dont la population totale est d'environ 25 millions d'habitants.

LE FOYER DE TRYPANOSOMIASE A *TRYPANOSOMA RHODESIENSE* AU BURUNDI*

LOUIS VAN DEN BERGHE†

Le Royaume du Burundi occupe un plateau montagneux d'axe Nord-Sud et d'altitude moyenne de 2.000 mètres environ constituant la crête de partage des eaux du Congo et du Nil. Les glossines vectrices de trypanosomiase n'existent que sur les flancs de plus basse altitude situés à l'Ouest, le long de la frontière de la République du Congo (Léopoldville) et à l'Est le long de la frontière de la République du Tanzania.

La trypanosomiase à *Trypanosoma gambiense* est seule présente dans les régions occidentales en certains points riverains du lac Tanganyika (Rumonge) et dans la vallée de la Ruzizi qui joint le lac Kivu au lac Tanganyika. Elle y est transmise par *Glossina fuscipes martinii*, race géographique plus tolérante que les autres *G. fuscipes* et surtout que *G. fuscipes fuscipes* à la sécheresse de l'air. La trypanosomiase à *T. gambiense* de l'Ouest du Burundi était quasi éradiquée en 1961 et ne représente pas de danger grave, les habitats naturels de *G. fuscipes martinii* (galeries forestières peu denses) tendant à disparaître et les habitats crées par l'homme (bananeraies) pouvant être aisément contrôlés.

La trypanosomiase à *T. rhodesiense* des régions orientales du Burundi constitue un problème autrement grave et difficile à résoudre. Venant des territoires du Tanganyika au cours de la progression de *G. morsitans* du Sud en Rhodesie vers le Nord jusqu'en Uganda, elle représente le flanc d'une invasion qui pourrait être suivie et datée sur cartes depuis quelques 50 ans. Nous avons estimé que c'est entre 1945 et 1950 que *G. morsitans* a franchi les galeries forestières orientales du Burundi pour penetrer vers l'Ouest à l'intérieur du pays. Peu de temps après, en 1954, les premiers cas d'infection à *T. rhodesiense* étaient signalés dans la province de Muhinga, puis en 1955 aussi dans la province de Ruyigi.

Nous extrayons du Rapport Annuel publié en 1962 par le Ministère de la Santé Publique du Royaume du Burundi les données suivantes concernant les nouveaux cas de trypanosomiase *rhodesiense* dans l'Est du Burundi.

	1954	1955	1956	1957	1958	1959	1960	1961	1962
Muhinga	97	57	114	11	18	34	26	36	14
Ruyigi		14	3	10		4		1	
Rutana				1	4	4	1		
Total	97	71	117	22	22	42	27	37	14

Le maximum d'intensité de l'épidémie fut atteint en 1956. Le traitement et la chimioprevention entrainèrent une chute très nette du nombre de cas nouveaux

* Cette esquisse épidémiologique a été effectuée dans le cadre d'une mission du Ministère de la Santé Publique du Royaume du Burundi. Nous adressons nos remerciements pour l'aide apportée à notre enquête par le Docteur Deviron, médecin directeur et Monsieur Bakire, directeur administratif de l'hôpital de Muhinga (Burundi) ainsi qu'aux trypanologues de l'EATRO à Tororo (Uganda) et des services spécialisés de Nairobi et Kisumu (Kenya) et de Bukoba (Tanzania). Nos remerciements sont adressés tout particulièrement à Monsieur Kahabuka, médecin directeur de l'hôpital de Biharamulo (Tanzania).

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en 1957 et 1958. Dès 1959 s'installait dans la province de Muhinga un foyer permanent à endémicité assez faible masquée cependant par une distribution très clairsemée de la population au demeurant très mobile de cette région frontière. Depuis 1960 les effectifs du Service Médical rural ayant été réduits on ne peut attacher aux nombres de cas nouveaux enregistrés en 1960, 1961 et 1962 la même signification qu'à ceux des années précédentes. En 1963 seize nouveaux cas ont été enregistrés par une Mission de Maladie du Sommeil à effectifs et moyens d'action très réduits. Il est probable que de nombreux cas ne sont pas venus à la connaissance du Service médical et il est possible que nous ayons en réalité une flambée épidémique au Nord de Muhinga qui s'apparente à celle que l'on observe actuellement autour du lac Victoria Nyanza. Il n'existe en effet aucune solution de continuité entre le foyer à *T. rhodesiense* installé en Burundi et celui qui occupe les districts de Ngara et de Biharamulo dans la West Lake région du Tanzania. Au surplus le lac Victoria Nyanza constitue une entité biologique et il est significatif d'observer que le phénomène épidémique dû à *T. rhodesiense* s'est produit au même moment, soit de fin 1963 à 1964, aussi bien au Sud-Ouest du lac à Biharamulo (Tanzania) qu'au Nord-Est du lac dans le district de Alego et dans le Lambwe Valley (Kenya).

Afin de dégager les données épidémiologiques essentielles du foyer du Burundi nous avons examiné les fiches cliniques réunies à l'hôpital de Muhinga de 84 malades représentant la quasi totalité des nouveaux cas de janvier 1960 à août 1964. Nous avons également visité les zones épidémiques du Kenya et du Tanzania et étudié l'histoire clinique de 151 cas (année 1964 jusqu'à octobre) réunis à l'hôpital de district de Biharamulo (Tanzania).

Les données épidémiologiques qui se dégagent de cette étude si on les limite au foyer du Nord-Est du Burundi sont les suivantes:

1. — La trypanosomiase à *T. rhodesiense* est bien établie au Nord-Est du Burundi, dans la province de Muhinga, plus particulièrement dans la région naturelle du Bugesera et aux abords de la rivière Ruvuvu là où elle contourne la colline frontière de Murama.

2. — Sur la base d'un foyer endémique établi depuis 1954 il semble que nous assistions actuellement à une réactivation du foyer, voire à une poussée épidémique contemporaine de celles observées en Tanzania et Kenya. L' actuelle difficulté de pratiquer un recensement médical valable ne permet pas l'établissement d'un taux correct de néo-infections. Les cas récents (1960 à août 1964) sont distribués également sur une surface de près de 1.200 kilomètres carrés. Le taux d'infectiosité des glossines est probablement assez faible, un seul membre d'une même famille étant le plus souvent atteint, alors que dans le district de Biharamulo (Tanzania) il est classique de trouver tous les membres d'une même famille atteints de trypanosomiase. Cependant la maladie affecte encore au Burundi un caractère professionel: chasse, cueillette d'eau et de bois, récolte de terre glaise le long de la Ruvuvu par les potiers Batwas.

3. — Les glossines paraissent très abondantes au Bugesera depuis deux à trois ans, de même que dans les savannes propices à l'Ouest du lac Victoria Nyanza. Il s'agit là peut-être d'un phénomène cyclique qui a souvent été évoqué mais il est évident que ces dernières années exceptionnellement pluvieuses ont favorisé la dispersion de *G. morsitans*. Des modifications très importantes de l'occupation des terres ont aussi permis la multiplication des glossines.

4. — La trypanosomiase à *T. rhodesiense* affecte une virulence très variable

dans son foyer du Nord-Est du Burundi. A côté de quelques rares cas très aigus, la majorité des cas présente un tableau clinique qui est plutôt classique de *T. gambiense* avec des antécédents torpides de plusieurs mois, un taux élevé de ganglions cervicaux (69 sur 84) et de ponctions lombaires (46 sur 84) positifs. Les observations faites dans les hôpitaux de Kisumu (Kenya) et Biharamulo (Tanzania) correspondent beaucoup plus au type *T. rhodesiense* de trypanosomiase. Les ganglions cervicaux n'y sont quasi jamais palpables. Il est possible que le dépistage des cas au Burundi ait favorisé la sélection des porteurs d'adénopathies. D'autre part on a reconnu de profondes transformations dans les caractères biologiques de *T. rhodesiense* qui le rapprochent de *T. gambiense*. La transmission de ces deux trypanosomes ne paraît plus étroitement liée non plus aux groupes *morsitans* pour *T. rhodesiense* et *fuscipes* pour *T. gambiense*. S'il apparaît clairement que *T. rhodesiense* est transmis par *G. fuscipes fuscipes* dans l'Alego district (Kenya) il ne semble pas non plus que l'existence de trypanosomiase humaine en zone *morsitans* par des glossines appartenant à ce groupe puisse exclure en principe la possibilité de *T. gambiense*.

Sur le plan de la lutte contre la trypanosomiase à *T. rhodesiense* au Burundi l'action doit être portée simultanément sur trois points:

a) la prospection et le traitement systématiques des cas,
b) la chémoprévention sélective,
c) le contrôle des vecteurs.

Pour ce troisième mode d'action la liaison avec les pays voisins faisant partie du même fly-belt et du même foyer endémique est indispensable.

RESUME

Une épidémie de trypanosomiase à *T. rhodesiense* a été découverte dans le Nord et l'Est du Burundi avec 97 cas en 1954. Le maximum de l'épidémie fut enregistré en 1956 avec 117 nouveaux cas tandis que seuls 14 cas nouveaux furent découverts en 1962 et 16 en 1963. La maladie paraît bien établie dans toute la zone à *G. morsitans* du Nord-Est du Burundi. Une analyse des données réunies pour 84 cas récents (1960 à 1964) dans la province de Muhinga permet de dégager certains faits épidémiologiques et de les comparer avec ceux des districts de Ngara et de Biharamulo en République du Tanzania.

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CONSIDERATIONS EPIDEMIOLOGIQUES SUR LA TRYPANOSOMIASE A *TRYPANOSOMA GAMBIENSE* DANS LA REPUBLIQUE DEMOCRATIQUE DU CONGO

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A. SITUATION DE L'ENDEMIE

Les derniers renseignements épidémiologiques complets datent de l'année 1958. Durant cet exercice, la quasi totalité des quelque 132 territoires du Congo avait été recensée du point de vue médical par les équipes itinérantes. Le nombre total des nouveaux cas dépistés s'élevait à 1.296 (statistique rectifiée) et l'indice moyen de contagiosité nouvelle était de 0,01 % avec des foyers résiduels où l'I.C.N. la plus élevée était de 0,4% (Lomami). De nombreuses opérations de chimioprophylaxie aux diamidines étaient encore en cours à la fin de l'année, notamment dans le Kasai. Certaines campagnes ont d'ailleurs pu être continuées pendant l'année suivante.

En 1959, les remous politiques et les luttes tribales avaient déjà fortement perturbé les activités de dépistage et de contrôle; seulement la moitié environ du pays a pu être couverte par les équipes itinérantes, mais tous les foyers résiduels et régions endémiques connus avaient néanmoins été visités sauf dans l'ex-province du Kasai pour laquelle les renseignements épidémiologiques font défaut. Le nombre total des nouveaux trypanosés s'élevait à 825. L'absentéisme était déjà fort important à ce moment dans les foyers visités et il est difficile d'établir des I.C.N. avec exactitude. L'impression générale était que l'endémie était stationnaire ou en légère régression.

Pour les années 1960 et 1961, les renseignements font pratiquement défaut. Les opérations de dépistage durant cette période se sont d'ailleurs limitées à quelques rares secteurs où des infirmiers ont tant bien que mal, de leur propre initiative, essayé de maintenir la routine du dépistage. Nous avons rassemblé quelques renseignements sur l'évolution de l'endémie, à posteriori, lors de nos prospections dans les principaux foyers:

Année	1960	1961	Région et Observations
Nouveaux Tryp.	15	20	Foyer Kimvula (frontière de l'Angola). Dépistage fait.
	2	3	Foyer Gombe Sud (frontière de l'Angola). Dépistage partiel.
	51	180	Foyer Tshofa (Lomami). Pas de dépistage en 1960. Dépistage partiel à partir du 2e trimestre 1961.

Pour l'année 1962, les renseignements étaient un peu plus amples et se rapportaient à une vingtaine de territoires où environ 20% de la population avait été examiné (soit environ 500.000 habitants au total). Le nombre de nouveaux

trypanosés dépistés s'élevait à 474. L'indice de contagiosité nouvelle accuse une hausse dans la plupart des foyers et atteint 0,5 % par endroits.

En détail:	foyer de Kimvula	22 N.T.
	foyer de Gombe Sud	6 N.T.
	foyer de Tshofa	146 N.T.
	foyer de Kibombo-Kasongo	80 N.T.

En 1963, nous avons constaté de nouveau un relâchement dans les activités itinérantes: 364 nouveaux trypanosés ont été renseignés pour une douzaine de territoires (population examinée: approximativement 300.000 habitants). L'indice de contagiosité nouvelle dans certains foyers dépasse 0,5 % et nous pouvons raisonnablement admettre qu'il atteint en beaucoup d'endroits 1 % car le dépistage n'est que partiel, l'absentéisme est fort élevé et les dispensaires diagnostiquent de nombreux trypanosés venant des villages non prospectés.

Quelques chiffres détaillés:

— foyer de Kimvula	99 Nouveau Trypanosés
— foyer de Gombe Sud	39 N.T.
— foyer de Tshofa	80 N.T. (partiel)
— foyer de Kibombo-Kasongo	97 N.T.
— foyer de Tulume (Kasai)	14 N.T.

Pour l'année 1964 nous ne disposons que des renseignements se rapportant aux huit premiers mois de l'année, pour les régions endémiques que nous avons prospectées en détail.

— Foyer de Kimvula: 178 nouveaux trypanosés, dont plus de 100 dans 6 villages du groupement Bangala, sur la frontière avec l'Angola (I.C.N. voisin de 3 %).
— Foyer de Gombe Sud: 68 N.T. (I.C.N. environ 1 %) dont beaucoup de réfugiés angolais. Récemment des trypanosés en provenance du secteur de Gombe Sud ont été dépistés à Léopoldville par les Services de l'Hygiène.
— Foyer de Tshikula (arrondissement de Dibaya dans le Kasai): 17 N.T.

Tableau des principaux foyers à trypanosomiase et du nombre de nouveaux trypanosés diagnostiqués de 1958 à 1964.

	1958	1959	1960	1961	1962	1963	1964‡
Kimvula* . .	12	11	15	20	22	99	178
Gombe Sud . .	79*	15	2	3	6	39	68
Tshofa (Lomami) .	114*	119	51	180†	146	80	?
Kibombo Kasongo .	53*	50	?	?	80	97	?
Tshikula (Kasai) .	22*	?	?	?	—	—	17
Tulume (Kasai) .	97*	12*	?	?	?	10	?
Gemena (Ubangi)* .	133	120	?	?	?	?	?

* Pentamidine.
† 51 N.T. diagnostiqués au dispensaire de Tshofa parmi les consultants ambulatoires, de mai à août 1961.
‡ 8 mois.

B. PROJET DE CAMPAGNE CONTRE L'ENDEMIE SOMMEILLEUSE

A la lumière de ces renseignements épidémiologiques, malheureusement trop sporadiques et parfois incomplets, il est indéniable que la maladie du sommeil regagne rapidement le terrain laborieusement conquis par des années d'efforts. Dès à présent, nous assistons à de véritables flambées épidémiques. Cet état de choses ne peut aller qu'en s'aggravant, étant donné que les difficultés pour maintenir en place les quelques rares équipes d'infirmiers itinérants routinés augmentent chaque jour. Dans le but d'entreprendre et de réorganiser une lutte efficace contre l'endémie sommeilleuse nous avons élaboré dès la fin de l'année 1963 un projet pour une " Campagne initiale de prospection et de lutte contre la trypanosomiase ". Ce projet, financé par le Fonds Médical Tropical en Belgique, sous la présidence du Professeur Dr Jansens, a reçu l'approbation du Gouvernement Congolais. Malgré la situation précaire du pays, nous avions dès juillet 1963 entamé la prospection préliminaire pour confirmer les renseignements concernant l'extension de l'endémie, évaluer la gravité de la situation, établir les priorités dans les opérations, ainsi que décider des mesures les plus adéquates à prendre pour l'éradication. Sur la base des renseignements épidémiologiques de 1958 et 1959, nous avons dressé une carte divisant le Congo en cinq secteurs endémiques qui couvrent ensemble un tiers du pays (les deux tiers restants pouvant être considérés avec une quasi certitude comme étant indemnes de trypanosomiase) et qui sont séparés plus ou moins par des barrières géographiques naturelles.

Ces secteurs sont respectivement:

1) Le Bas-Congo, compris entre l'Atlantique et la rivière Kwango-Kwa.
2) Le Kwango-Kwilu, compris entre les rivières Kwango et Kasai.
3) Lac Léopold II — Cuvette Centrale, compris entre le fleuve Congo et le Kwa-Kasai.
4) (*a*) — L'Ubangi — Moyen Congo, compris entre le fleuve Congo et la rivière Ubangi.
   (*b*) — Un petit secteur en bordure de la frontière du Soudan.
5) Le Kasai — Maniema (mal délimité par des barrières naturelles).

Dans chaque secteur nous entreprenons une prospection approfondie afin d'étudier en détail tous les facteurs épidémiologiques qui sont responsables de l'entretien des foyers résiduels ou des flambées épidémiques que nous constatons.

Pour l'exécution des campagnes, nous avons prévu la constitution d'Unités Opérationnelles Mobiles (une ou plusieurs par secteur si nécessaire) qui procéderont à des " Opérations Combinées " de dépistage, contrôle, traitement, lutte contre le vecteur par piégeage et fogging d'insecticides résiduels. C'est précisément dans le but de réduire les frais des opérations de lutte contre le vecteur que nous insistons sur l'étude minutieuse du foyer endémique afin de le délimiter le plus exactement possible et de localiser les points névralgiques de contact homme/mouche.

Nous avons prévu un car-laboratoire comme élément indispensable pour les Unités Opérationnelles Mobiles, bien qu'à ce moment, faute d'informations, nous ignorions les possibilités pleines de promesses, de recherche des hyper-bêta-2-macroglobulinémies (Communication du Médecin-Général-Inspecteur P. Richet, p. 173). Ces Unités Mobiles devront s'intégrer dans le cadre de la lutte en général contre les grandes endémies. Elles seront donc en principe polyvalentes mais à notre avis cette polyvalence ne devra pas être " simultanée " mais " échelonnée "

dans le temps, c'est-à-dire qu'elle s'occupera uniquement de la trypanosomiase, dans la phase opérationnelle de la campagne, quitte à effectuer par exemple des vaccinations B.C.G. pendant la phase de contrôle.

C. CHIMIOPROPHYLAXIE AUX DIAMIDINES

Dans le cadre des mesures de lutte contre la trypanosomiase, nous avons délibérément omis de mentionner la chimioprophylaxie, car nous n'entendions l'employer que dans certains rares cas bien déterminés et après avoir acquis la certitude que le virus circulant soit réduit à un minimum. En effet, la prophylaxie à la Propamidine, Pentamidine et Lomidine, qui a été utilisée dans tous les foyers à trypanosomiase au Congo depuis 1946, qui fut poursuivie pendant de nombreuses campagnes et répétée jusqu'à 6 et 8 injections semestrielles, n'a pas donné les résultats durables qu'on en attendait. Avec le recul que nous possédons actuellement, elle peut même, dans de nombreux foyers, être considérée comme un échec, à moins d'avoir pris des précautions ainsi que nous l'avons fait en 1949 dans un essai d'éradication d'un foyer hyperendémique au Kwilu. Ces mesures préliminaires aux séances d'injection du chemoprophylactique consistaient à pratiquer deux dépistages exhaustifs consécutifs à un mois d'intervalle en employant pour le diagnostic la ponction ganglionnaire associée à la prise de gouttes épaisses de sang généralisées, la ponction sternale et lombaire chez les suspects et même, comme nous l'avons pratiqué dans les villages les plus infestés, la ponction lombaire généralisée à toute la population. Il semblerait à l'heure actuelle que la méthode de recherche des hyper-bêta-2-macroglobulinémies peut remplacer avantageusement toutes ces mesures de précaution en multipliant par soixante les chances de détection des trypanosés (communication à cette réunion du Médecin-Général-Inspecteur P. Richet).

Il n'en reste néanmoins qu'il faut en outre étendre la prophylaxie aux groupements de populations saines autour du foyer, en zone non endémique, de façon à créer une zone-tampon. Cette zone doit autant que possible être étendue jusqu'à la limite de barrières naturelles. Douze ans après cette expérience aucun nouveau trypanosé n'avait encore été dépisté dans le foyer ainsi traité. Des conditions indépendantes de notre volonté ne nous ont pas encore permis de contrôler si les résultats se maintiennent. Il serait extrêmement intéressant de pouvoir vérifier par la recherche des hyper-bêta-2-macroglobulinémies.

D. CONSIDERATIONS SUR L'EVOLUTION DE LA TRYPANOSOMIASE-MALADIE

Nous voudrions mettre l'accent sur la difficulté à interpréter les facteurs épidémiologiques en cause dans certaines flambées épidémiques de trypanosomiase. Au cours de nos récentes prospections dans les anciens foyers résiduels, nous avons été confrontés avec l'apparition brusque, et quasi simultanée, d'un certain nombre de trypanosés à un stade d'évolution extrême de la maladie, dans une région où depuis cinq ans aucun trypanosé n'avait été diagnostiqué.

Cette région, autour de Tshikula (territoire Dibaya, province de Luluabourg), est située à la limite d'un ancien foyer de trypanosomiase éteint en 1958 par la chimioprophylaxie. De mars à juillet 1964, 17 nouveaux trypanosés ont été amenés au dispensaire de l'hôpital de Tshikula, pour des troubles mentaux, hémiplégie,

fièvres, etc. Les trypanosés étaient originaires de différents villages éparpillés dans un rayon de 12 km environ autour de Tshikula. Il nous est difficile d'admettre, quoique l'état avancé des malades semble le prouver, qu'il s'agit d'une infection remontant à un an ou dix-huit mois comme on pourrait s'y attendre en présence d'une infection à *T. gambiense* au stade de méningo-encéphalite avancée.

Quoiqu'il n'y ait pas eu de dépistage pratiqué par une équipe itinérante, nous avons constaté dans d'autres foyers où le dépistage avait également été abandonné, que régulièrement des cas de trypanosomiase à des stades divers d'évolution étaient diagnostiqués dans les dispensaires. Normalement l'hôpital de Tshikula aurait dû diagnostiquer un certain nombre de trypanosés à partir du début de l'année 1963 si l'on conclut à un réveil normal d'un foyer résiduel. Nous opinons plutôt pour une reprise de l'endémie avec une évolution nerveuse accélérée se rapprochant du tableau clinique de l'infection à *T. rhodesiense*. Nous voudrions également mettre cette observation en rapport avec nos observations recueillies de 1946 à 1956, dans la zone Foreami, et qui nous avaient amenés à postuler une évolution plus rapide vers le stade nerveux des trypanosés dans les zones endémiques qui avaient été soumises à la prophylaxie chimique quelques années auparavant.

Tout en ayant gardé le même rythme (semestriel) de dépistage, et employé les mêmes méthodes d'examen et pratiquement la même équipe, nous avons constaté (statistique 1955) qu'au lieu de dépister 80% à 90% des trypanosés par la ponction ganglionnaire et l'examen de sang, nous obtenions les résultats suivants:

Mode de diagnostic:	Ponction ggl.	Ex. sang	Ponct. ster.	L.R.C.
% de N.T.:	40%	17,8%	2,2%	40%

Le nombre de nouveaux trypanosés diagnostiqués à la période de méningo-encéphalite avait évolué dans les mêmes proportions.

Nous avons pu vérifier ces mêmes observations récemment dans les foyers du Lomami et de Kimvula. Ces deux dernières observations concordent parfaitement quoique dans le foyer du Lomami le dépistage ait été interrompu en 1960 et par la suite effectué sporadiquement, tandis que dans le foyer de Kimvula (région ayant appartenu au Foreami) le dépistage a été continué régulièrement.

Etat du L.C.R. au moment du diagnostic

Nombre d'éléments	0–3	9–20	20–100	100 et plus	Total
KIMVULA					
Nombre de P.L. faites . .	65	112	45	116	338
Pourcentage	19%	33%	14%	34%	100%
LOMAMI					
Nombre de P.L. faites . .	64	127	142	128	461
Pourcentage	14%	28%	30%	28%	100%

Le nombre de malades diagnostiqués au stade lymphatico-sanguin (0-3 éléments) est respectivement de 19% et 14% avec un léger avantage pour la zone où le dépistage a été régulier.

Le pourcentage des altérations liquidiennes est respectivement de 81% et de 86%.

Parmi les malades à L.C.K. altéré, respectivement 33% et 28% se trouvent en période de réaction méningée.

Respectivement 48% et 58% de nouveaux trypanosés diagnostiqués sont en période de méningo-encéphalite dont 34% et 28% à un stade avancé.

E. FACTEURS QUI ENTRETIENNENT L'ENDEMIE

Parmi les facteurs qui entretiennent l'endémie et qui relèvent de l'homme, par le fait qu'ils contribuent à maintenir le réservoir de virus, nous avons constaté au cours de nos prospections des dernières années que ces facteurs sont principalement:

1) Erreurs grossières dans le choix du trypanocide ou dans le schéma de traitement.
2) Mise en traitement sans ponction lombaire préalable.
3) Irrégularité ou interruption du traitement (malade indiscipliné).
4) Absence de contrôle régulier par examen périodique du L.C.K. chez les malades mis en guérison provisoire, maintenue ou même confirmée.

Si nous établissons une comparaison entre les résultats du traitement et l'évolution ultérieure chez les trypanosés des foyers de Kimvula et du Lomami d'une part, et les normes établies par le Foreami d'autre part, nous constatons que:

— le nombre de guérisons obtenues au Foreami était de 85% dans la première année (70% de guérisons après une première cure, plus 50% de guérisons parmi les malades soumis à une deuxième cure);
— au Lomami nous enregistrons un échec thérapeutique, ainsi que nous l'avons constaté à la suite d'erreurs de traitement et aussi par manque de trypanocides appropriés: seulement 47,6% de guérisons (soit 27,6% de malades guéris et 20,0% en voie de guérison);
— à Kimvula, les résultats sont meilleurs mais encore loin en dessous de la norme: 64% de guérisons (soit 44% de malades guéris et 20% en évolution favorable vers la guérison). La cause des échecs est due principalement aux malades indisciplinés (population flottante des régions frontières).

Les statistiques du Foreami renseignent 5 à 6% de malades évoluant vers le stade chronique. Au Lomami et à Kimvula, nous enregistrons respectivement 25% et 13% de malades chroniques. Outre les erreurs de traitement, il y a eu manifestement un manque de contrôle.

La litalité parmi les malades du Foreami était de 5%. Elle est respectivement de 8% et de 9% au Lomami et à Kimvula.

Un des facteurs principaux de l'entretien de l'endémie sont les malades qui se dérobent au traitement et aux contrôles, ceux qu'on appelle communément " les disparus " et qui constituent de véritables réservoirs de virus.

Les " disparitions " renseignées par le Foreami étaient de 4%. A Kimvula, la proportion de " disparus " est de 1 malade sur 7 (14%).

%	Foreami	Kimvula	Lomami
Guérisons	85%	64%	48%
Litalité	5%	9%	8%
Disparitions	4%	14%	19%
Malades chroniques	6%	13%	25%

FINAL REPORT ON A FIELD TRIAL OF MEL.W. IN THE TREATMENT OF GAMBIAN SLEEPING SICKNESS

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A preliminary report on this trial was given to the ISCTR Meeting at Conakry in 1962; the present report covers the whole follow-up period of three years and the conclusions reached.

At the second follow-up, twenty-three months post-treatment, seven patients were found to have signs of deterioration (Watson, 1962), so it was decided to re-examine them again after six months, in case further treatment was needed. At this re-examination they were all found to have improved considerably and no further treatment was ordered (see Table I).

Table I

Patient No.	March 1962			October 1962		
	E.S.R.	C.S.F. Cells	C.S.F. Protein	E.S.R.	C.S.F. Cells	C.S.F. Protein
33 (E) . .	15–40	1	33	10–38	1	9
37 (E) . .	13–32	0	32	5–19	4*	24
7 (I) . .	6–21	3	42	6–21	2	21
8 (I) . .	10–29	1	41	11–29	2	30
27 (I) . .	23–55	2	29	8–24	2	15
15 (A) . .	7–21	10	25	5–12	2	10
23 (A) . .	21–53	3	46	7–19	0	36

* Some blood staining. E = Early case. I = Intermediate case. A = Advanced case.

At the final follow-up, thirty-six months post-treatment, thirty-six of the original thirty-eight patients were examined, this included the patient who absconded before having completed his treatment; two advanced cases were absent. One of these patients was reported to be well, the other to have died; death in this case was, almost certainly, associated with sleeping sickness. She had been reported in 1962 as being mentally deranged and roaming the country.

In one case the C.S.F. cell count was raised; however, the presence of red blood corpuscles in the fluid indicated that there was blood contamination of the sample. The findings for the C.S.F. in this patient (No. 4) at the final follow-up are, therefore, invalid as the patient failed to attend for another lumbar puncture. The C.S.F. protein content had risen in some cases, notably No. 37, in whom it was 38 mgm./100 ml.; other findings in this patient were C.S.F. cells 1/cmm., E.S.R. 5–15, Serum Protein 7·2 g./100 ml. and Haemoglobin 116% Haldane. As the patient reported that he was well and strong, did not sleep in the day-time and only occasionally had a headache, it is considered that he has been cured. Other patients in whom the C.S.F. protein content had risen also reported that they were well, and, in the absence of other adverse findings, they also were considered to be cured.

Table II.—C.S.F. Findings

EARLY CASES

Patient No.	1960 Cells	1960 Protein	1961 Cells	1961 Protein	1962 Cells	1962 Protein	1963 Cells	1963 Protein
2	2	14	0	9	1	19	1	17
5	13	10	1	8	2	20	1	12
9	2	14	1	7	1	15	–	–
10	3	12	2	23	5	14	1	15
12	5	10	3	7	9	16	1	24
13	3	12	1	14	0	19	1	20
16	6	14	1	14	A	A	0	21
20	8	12	1	18	1	18	1	28
21	1	10	4	17	1	17	0	22
24	2	10	1	13	1	13	1	18
29	1	13	1	16	A	A	2	30
32	1	12	A	A	A	A	1	21
33	9	11	2	16	1	33	1	22
37	2	15	1	18	0	32	1	38
Mean	4·4	12·07	1·46	13·8	2·09	22·2	0·9	20·8
S.D.	3·63	1·73	1·05	5·15	2·6	6·63	0·54	5·23

INTERMEDIATE CASES

Patient No.	1960 Cells	1960 Protein	1961 Cells	1961 Protein	1962 Cells	1962 Protein	1963 Cells	1963 Protein
1	17	16	1	5	3	21	2	23
3	25	10	1	8	1	12	0	24
4	38	28	1	15	1	24	12*	34*
7	16	25	1	16	3	42	1	30
8	1	39	5	21	1	41	1	25
11	19	15	1	8	1	13	0	13
14	2	33	0	20	2	22	0	25
26	35	20	A	A	2	20	1	28
27	32	18	2	13	2	29	1	21
28	35	21	2	17	0	22	2	24
30	11	12	0	8	0	11	2	17
34	12	24	0	24	2	20	3	26
Mean	20·25	21·75	1·28	14·09	1·5	23·1	2·08	24·2
S.D.	12·8	8·6	1·42	6·24	1·0	10·0	3·25	5·38

LATE CASES

Patient No.	1960 Cells	1960 Protein	1961 Cells	1961 Protein	1962 Cells	1962 Protein	1963 Cells	1963 Protein
6	6	42	2	35	4	19	1	34
15	58	24	3	13	10	25	0	21
17	172	63	1	16	1	17	A	A
18	55	31	0	12	1	22	0	32
19	400	62	4	36	5	35	2	44
22	660	62	9	18	2	23	0	23
23	1040	102	4	42	3	46	0	39
25	595	51	2	21	5	28	1	36
31	45	22	3	21	1	20	1	31
35	300	26	A	A	1	19	3	31
36	530	50	Excluded from follow-up examinations					
38	115	58	5	32	A	A	A	A
Mean	331	49·42	3·3	23·6	3·3	25·4	0·9	32·9
S.D.	321	12·4	2·49	9·97	2·86	28·2	0·316	7·03

"A": denotes absentee.

In a number of late cases, the C.S.F. protein content had not returned to normal, although the cell count had done so and all other indications were that the patient had been cured; it would appear that there had been some permanent damage to the blood brain barrier in these cases and that the C.S.F. protein will remain raised.

The indications from this field trial are that, in the regimen used, Mel.W. has a definite place in the treatment of sleeping sickness due to *T. gambiense*.

Reference

Watson, H. J. C. (1962). A field trial of Mel.W. in the treatment of Gambian sleeping sickness. ISCTR Ninth Meeting, Conakry.—CCTA Publication No. 88, p. 347.

ANALYSIS OF THE DEVELOPMENT OF ARSENICAL RESISTANCE IN TRYPANOSOMES

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(Abstract of Paper)

A homogeneous strain of trypanosomes, such as an old laboratory one, is conceived as a population of individuals with a normal distribution of sensitivity to drugs around a mean value. This sensitivity may be measured as the concentration of drug required to kill trypanosomes in a given time; and the characteristics of the population can be expressed as the mean value and the standard deviation. In a heterogeneous strain (such as a freshly isolated strain, or one which is undergoing modification) the strain would be several such populations. A method has been devised (by P. J. Walker) so that populations differing in resistance to arsenicals and to acriflavine can be analysed. Briefly, after incubation in graded concentrations of acriflavine, the trypanosomes are exposed on a microscope stage to a standard illumination for ten seconds. The percentage of trypanosomes which have been killed is counted. The probit of the percentage killed is plotted against log concentration of acriflavine. If a straight line results, it indicates a single population of which the mean value and standard deviation can be calculated. If the slope is broken by one or more level parts, it indicates two or more populations with estimates of their proportions and their degree of resistance. The methods were checked by testing them upon artificial mixtures of normal and resistant trypanosomes.

EXPERIMENT

In order to study the development of drug resistance, an old laboratory strain of *T. brucei* has been exposed to trivalent tryparsamide *in vitro*, so as to select out the more resistant individuals, and then the suspensions have been injected into rats. The rats which became infected after the longest prepatent periods (i.e. infected with a few of the most resistant trypanosomes) were selected for further passage; and so on, repeatedly. After each selection the composition and resistance of the strain were examined. This process of repeated selection was carried out on five substrains which were initially all identical being derived from the same original strain.

RESULTS

(1) Increased resistance was never obtained after the first selection *in vitro*; the trypanosomes which survived the first selection reproduced a population no different from the original one. Therefore the original strain did not contain resistant individuals (selection at 1 in 10^7).

(2) Resistance (when it did occur) appeared at different times in different strains, e.g. after two selections in Strain E and after twelve selections in Strain B.

Presumably such resistance was due to the spontaneous appearance of resistant mutants, which were then selected out for propagation at the next exposure to drug *in vitro*.

(3) Five different " levels " of resistance could be distinguished (in addition to the original sensitivity), i.e. when resistance appears, the mean value of the population does not shift gradually, but only by a (limited) number of steps, each presumably corresponding to some different arrangement of the genetic material of the trypanosome. In the present complex, the two highest levels of resistance were not stable and the populations at these levels usually constituted less than 40% of the complete strain. In one strain (B), the main population persisted at the lowest of these levels of resistance (photosensitivity units, 5·9) even after twenty selections. It would be possible to explain the five levels observed by postulating three genes which might interact in different ways.

It is suggested that other modifications of trypanosomes (relating to virulence, morphology, biochemistry, antigenicity, etc.) might well be explained in the same way, viz. that for each character there are a number of possible populations, and that the change from one population to another is discontinuous. The apparent characters of the complete strain would thus depend upon the relative proportions of the different populations present at some particular moment. Old laboratory strains are usually homogeneous because they have been selected over a long period according to the single character for rapid multiplication in laboratory animals. Freshly isolated strains comprise many different populations, adapted to the varying conditions in the fly and mammal through which such strains must pass.

DIAGNOSIS OF HUMAN SLEEPING SICKNESS IN MEDICAL UNITS WITH LIMITED LABORATORY FACILITIES

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INTRODUCTION

During an outbreak of human sleeping sickness, a number of suspects with pyrexia of unknown origin are generally sent to rural medical units or district hospitals in order to establish the precise diagnosis. Also, in epidemiological studies, the incidence of the disease in an endemic area can only be accurately estimated when the number of infected persons is precisely known. In both situations it is necessary to examine a large number of people within a limited period. The application of the array of modern diagnostic methods to these people is fraught with many difficulties. Cultural methods, although reliable (Weinman, 1960) are liable to contamination and require storage at a controlled temperature. Inoculation of blood into suitable laboratory animals requires a very elaborate organisation and adequate manpower and the apparatus necessary for the operation is not usually available in these small rural medical units because of expense. Examination of gland juice is complementary to blood film examination but, by itself, is not a reliable test because, in subclinical cases, glandular enlargement does not occur (Shore, 1960). A serological (agglutination) test (Cunningham, 1960) will detect long-standing cases of human trypanosomiasis but will not distinguish treated from untreated cases. The test is promising, but its clinical application has not been fully investigated. Further, at this stage, multiple antigens are still necessary for the proper conduct of the test. Thus, in the absence of a satisfactory method for the diagnosis of cryptic trypanosomiasis, the conventional method of examining stained blood films remains the simplest and most easily applied test under " field " conditions. It has, however, two major drawbacks. First, it requires a diligent and experienced microscopist, and secondly, in cases of low parasitaemia, a positive finding is a matter of mere luck because films known to be positive have been found negative even after 1,000 microscopic fields have been carefully examined (Cunningham and van Hoeve, 1964).

The authors wish to record a preliminary report on observations on a patient and to demonstrate that daily examination of blood films, extended for a period of over a week, is likely to reduce the existing degree of error in this method of diagnosing human sleeping sickness due to *T. rhodesiense*. Parallel with the investigation aimed at testing the efficiency of blood film examination as a diagnostic tool, the simultaneous daily titration of the patient's blood in order to measure the number of infective trypanosomes was carried out.

MATERIALS AND METHODS

An adult male patient, aged forty, a fisherman by occupation, was admitted into the EATRO Hospital infected with *T. rhodesiense*. On admission, he gave a history of having been ill with fever and headache for a fortnight only. His general condition

was fairly good; he was well nourished, with a clean, glossy skin; there was no evidence of oedema or of anaemia. He weighed 63·0 kg.; his temperature was 99·4° F., pulse rate 68 per minute and blood pressure 120 systolic over 80 diastolic. On physical examination his spleen was palpable two fingers, but was neither soft nor tender. Two hard and painless posterior cervical glands, one on each side and measuring the size of a pea, were palpated. The femoral and inguinal glands were enlarged but not tender. Nothing abnormal was found in the respiratory and cardio-vascular systems. Central nervous system examination revealed weak knee and ankle jerks and a normal plantar flexor response was demonstrable. Sensation to touch, pain and temperature were normal. C.S.F. examination showed nine w.b. cells, 18 mgm% of proteins and no trypanosomes. Gland juice, stained thick blood film and wet blood film preparations were all positive for trypanosomes. In view of his good general condition and early stage of infection, it was decided to keep him under observation on placebo treatment for three weeks. Daily temperatures and pulse rates were recorded four-hourly. No antipyretic or analgesic was administered. During this period, daily microscopical examination of his blood for parasites and daily titration in mice to determine the number of infectious organisms (Lumsden *et al.*, 1963) present were undertaken.

RESULTS

The following results were obtained and are shown in the table:

(1) Trypanosomes were found on the day of admission and also on day 1, 8, 15, 16, 17 and 19 after admission by blood film examination. No trypanosomes were seen on any other days. The microscopist was not informed of the nature of investigation.

Days after admission	Stained blood film examination	Highest temperature (on each day)	Highest pulse rate (on each day)	Trypanosome infectivity (log. No. ID_{63}/ml.)
0	+	99·4° F.	80	Not done
1	+	99·0° F.	80	1·4 + 0·5
2	−	98·0° F.	80	0
3	−	97·2° F.	60	1·6 ± 0·3
4	−	98·0° F.	64	2·8 ± 0·3
5	−	98·0° F.	68	3·8 ± 0·4
6	−	99·0° F.	62	5·0 ± 0·3
7	−	103·8° F.	84	5·4 ± 0·5
8	+	100·0° F.	76	4·7 ± 0·3
9	−	101·2° F.	80	1·8 ± 0·3
10	−	98·6° F.	68	0
11	−	96·8° F.	58	0
12	−	97·4° F.	60	0
13	−	98·0° F.	70	2·4
14	−	97·8° F.	68	3·4 ± 0·5
15	+	99·2° F.	80	4·4 ± 0.3
16	+	99·0° F.	76	2·8 ± 0·5
17	+	98·0° F.	70	2·1 ± 0·5
18	−	98·0° F.	72	1·8 + 0·3
19	+	98·0° F.	64	1·8 + 0·3
20	Not done	97·8° F.	62	0
21	Not done	97·6° F.	64	0

(2) The temperature chart illustrated above shows two peaks which coincide with peaks of parasitaemia as shown by the infectivity titrations.
(3) Changes in the pulse rate followed closely the changes in temperature.
(4) Infectivity titration showed that the number of infective organisms varies from day to day, increasing and decreasing as the temperature rises and falls.

SUMMARY AND CONCLUSIONS

This experiment shows that rises in temperature during an infection with *T. rhodesiense* occur at intervals of about a week. These rises coincide with an increase in the number of trypanosomes infective to mice. This finding is similar to that of Ross and Thomson (1910) whose observations were made on a patient infected in Zambia probably by the same species of trypanosome. These workers also found, by a different method, that the rise and fall in numbers of trypanosomes in the peripheral blood, coincided with the rise and fall in the patient's temperature.

While animal inoculation is clearly the more sensitive method of diagnosis, as pointed out above, it is unlikely to be used as a diagnostic tool in rural medical units and in the majority of surveys where the simpler method of examination of blood films will be the one normally used. From the result of this experiment it is clear that such examination, where the films are negative, should be carried out daily for at least one week and that extra diligence should be exercised during the periodic rise of temperature as there is a distinctly greater chance of finding trypanosomes at this time.

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THE ESTIMATION OF THE CONCENTRATION OF THE IgM CLASS OF IMMUNOGLOBULINS IN THE SERUM AS AN AID TO THE DIAGNOSIS OF TRYPANOSOMIASIS IN MAN

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INTRODUCTION

The value of tests for indicating a raised IgM immunoglobulin content in the serum as an aid to the diagnosis of trypanosomiasis in man has been amply demonstrated by the comprehensive survey of the work recently carried out in French-speaking West Africa presented to this meeting by Médecin Général Inspecteur Richet (see p. 173). The present paper summarises, preliminarily, work done under the auspices of the World Health Organisation and in collaboration with the East African Trypanosomiasis Research Organisation. The excellent assistance afforded to me by these two organisations has rendered the work possible. I should like to acknowledge, also, the help given to me by my colleagues in the Department of Bacteriology, University of Edinburgh.

NOMENCLATURE

For the sake of clarity in discussion the three main classes of human immunoglobulins, as proposed by the WHO meeting in Prague in June 1964, together with other usages and information are as follows:

Proposed usage	Previous usages	Approximate molecular weight
IgG	γ, 7S, γ ss	160,000
IgA	β_2A, γ_1A	
IgM	γ_1M, β_2M, 19Sγ, γ macroglobulin	900,000

The recognition of the great increase in the IgM component (β_2 macroglobulin) in trypanosomiasis in man and the indication of the importance of measuring the increase as an aid to the diagnosis of human trypanosomiasis, is due to Mattern and his colleagues (e.g. Mattern, P., Masseyeff, R., Michel, R., and Peretti, P., 1961, *Ann. Inst. Pasteur*, **101**, 382–8).

METHODS

Although there is evidence that the increase in IgM immunoglobulins in trypanosomiasis is a specific response to the infection the basis of the present approach is simply a quantitative one, that the increase in IgM immunoglobulins in the serum in trypanosomiasis is greater than in other disease conditions. Methods are required, therefore, for the quantitative estimation of IgM immunoglobulins in the sera of the persons under study. There are three main methods for this purpose, all based on the precipitation of antigen-antibody complexes in agar-gel:

(*a*) The serum to be tested is placed in one well and an anti-serum specific to IgM placed in an adjacent well. The position subsequently occupied

by the precipitation zone, along the line joining the two wells is influenced by the relative concentrations of antigen and antibody and measurement of its position can be used to indicate the amount of IgM present.

(*b*) The serum to be tested may be diluted serially and the several serial dilutions inserted in successive wells, each opposed to a well containing anti-serum specific to IgM. The point at which the precipitation line disappears is indicative of the amount of IgM present.

(*c*) The anti-serum specific to IgM is incorporated in the agar-gel and the sera under test are introduced individually in wells in the gel. The diameter of the circular area of precipitation developing round a well is indicative of the amount of IgM present in the specimen.

Present studies deal with the last method. Suitable plates are commercially available (Hyland Laboratories, Los Angeles, California).

RESULTS

The serum of a patient with no indication of any trypanosome infection and the sera of five patients proven infected with *T. rhodesiense* were each tested eight times, each time on a different plate. The results obtained were acceptably consistent and the ranges of the " infected " and " non-infected " sera did not overlap.

A more extensive study of known " infected " sera was made. The results were compared with those obtained with the sera of patients under treatment for visceral leishmaniasis, tuberculosis, leprosy and with sera from persons investigated in a recent survey of the sleeping sickness area in Alego, Central Nyanza, Kenya, who gave no indication of trypanosome infection by slide examination of the blood, by the inoculation of blood into mice and into culture and by agglutination testing with six antigens. Of the fifty sera not associated with any parasitological or immunological evidence of trypanosome infection, six gave results falling within the lower part of the range expected from sera of persons infected with trypanosomiasis. The possibility that the donors of these sera had been or were infected with trypanosomiasis cannot be excluded and intensive parasitological and immunological investigations of such persons is indicated.

Work in Edinburgh on the fractionation of antisera to trypanosomes prepared in rats, by means of centrifugation on sucrose gradients, showed that the main agglutinating activity of the sera resides in the same fractions as those which contain the IgM immunoglobulins.

LIST OF WORKING DOCUMENTS
LISTE DES COMMUNICATIONS

Document No. ISCTR (64)	*Title/Titre*	*Author/Auteur*
1	Draft Agenda/Projet d'Ordre du Jour	
2	Final Report on a field trial of Mel.W. in the treatment of Gambian sleeping sickness . . . . Rapport final sur un essai effectué sur le terrain avec le Mel.W. pour le traitement de la maladie du sommeil à *T. gambiense*	H. J. C. WATSON
3	Long-term fluctuations in numbers of a population of *G. palpalis palpalis* (R.-D.): Sixteen years' observations . . . . . Fluctuations numériques à long terme dans une population de *G. palpalis palpalis* (R.-D.): Seize années d'observations	A. M. JORDAN
4	An experiment on the eradication of *G. swynnertoni* Aust. by insecticidal treatment of the resting sites Expérience d'éradication de *G. swynnertoni* Aust. par traitement insecticide des gites	P. R. CHADWICK, J. S. S. BEESLEY, P. J. WHITE & H. T. MATECHI
5	Aerial application of insecticides in East Africa. XIV.—Very-low-volume aerosol applications of Dieldrin and Telodrin for the control of *G. morsitans* Westw. . . . . Applications aériennes d'insecticides en Afrique Orientale. XIV. — Applications d'aérosol de Dieldrine et de Télodrine en très petits volumes dans la lutte contre *G. morsitans* Westw.	G. F. BURNETT, P. R. CHADWICK, A. W. D. MILLER & J. S. S. BEESLEY
6	The biological control of the antigenic characters of a strain of trypanosomes . . . . . Contrôle biologique des caractères antigènes d'une souche de trypanosomes	A. R. GRAY
7	La trypanosomiase humaine résiduelle à *T. gambiense* en Afrique francophone de l'Ouest . . Residual human trypanosomiasis *T. gambiense* in French-speaking West Africa	P. RICHET
8	Report on results from insecticidal control projects for the eradication of *Glossina* in the Sudan vegetational zone in Northern Nigeria . . . . Rapport sur les Résultats du Projet de Contrôle Insecticidal pour l'Extermination des Glossines dans la Zone Végétationnelle soudanaise du Nigéria du Nord	K. J. R. MACLENNAN
9	Seasonal variations in age and infection rate of *G. fuscipes* . . . . . Variations saisonnières dans l'âge et l'index de contamination des glossines *fuscipes*	J. M. B. HARLEY
10	Some observations on the treatment of cattle with Berenil . . . . . Quelques observations sur le traitement du bétail au Bérénil	K. VAN HOEVE & M. P. CUNNINGHAM
11	Separation of trypanosomes from rat blood components . . . . . Séparation de trypanosomes des composantes sanguines du rat	V. SIMMONS, R. H. KNIGHT & K. C. HUMPHRYES
12	The duration of infectivity to mice of trypanosomes ingested by *G. pallidipes* . . . . Durée de la contagion transmise par trypanosomes ingérés aux souris	M. P. CUNNINGHAM & J. M. B. HARLEY

Document No. ISCTR (64)	*Title/Titre*	*Author/Auteur*
13	Etude de l'effet de l'HCH nébulisé sur une population de *G. palpalis gambiense* Vanderplank 1949 dans une galerie forestière (Kankalaba, République de Haute-Volta) . . . . . Study of the effect of atomised HCH on a population of *G. palpalis gambiense* Vanderplank 1949, in a riverine forest (Kankalaba, Republic of Upper Volta)	A. CHALLIER, M. EYRAUD & B. DEDEWANOU
14	Diagnosis of trypanosomiasis in cattle . . . . Diagnostic de la trypanosomiase affectant le bétail	M. P. CUNNINGHAM & K. VAN HOEVE
15	Essais des médicaments trypanopréventifs chez les ânes . . . . . . Experiments with anti-trypanosomiasis prophylactic drugs on donkeys	P. FINELLE & R. LACOTTE
16	Rapport sur les progrès récents de la chimiothérapie des trypanosomiases animales . . . . . Report on recent progress in the chemotherapy of animal trypanosomiasis	P. FINELLE
17	Diagnosis of human sleeping sickness in medical units with limited laboratory facilities . . Diagnostic de la maladie du sommeil dans des centres médicaux ne disposant que le laboratoires réduits	R. J. ONYANGO & P. DE RAADT
18	List of documents issued for the meeting Liste des documents diffusés pour la réunion	
19	Utilisation des glucides et de leurs produits de métabolisme par *T. evansi* et *T. brucei* . . The utilisation of glucosides and their metabolism products by *T. evansi* and *T. brucei*	J. BALIS
20	Elimination de l'acide pyruvique des milieux de culture en vue de favoriser la survie de *T. evansi* Elimination of pyruvic acid from culture media to improve the survival of *T. evansi*	J. BALIS
21	Applications par voie aérienne de Télodrine dans la lutte contre *G. morsitans* Westw. au Bugesera (Rwanda) . . . . . . Aerial application of Telodrin in the control of *G. morsitans* Westw. in Bugesera (Rwanda)	E. J. BUYCKX
22	Prophylaxis and suppression of trypanosomes by chemotherapeutic compounds with reference to drug resistance and regimes . . . . . Prophylaxie des trypanosomiases et suppression des trypanosomes par l'emploi des composés chimiothérapiques, avec référence particulière à la chimiorésistance et les régimes	M. J. HOPE CAWDERY
23	Analysis of the development of arsenical resistance in trypanosomes . . . . . . Analyse de l'apparition et de l'évolution de la résistance aux médicaments arsenicaux dans les trypanosomiases	F. HAWKING & P. J. WALKER
24	The eradication of *G. morsitans morsitans* Westw. in Ankole, Western Uganda, by Dieldrin application Eradication de *G. morsitans morsitans* Westw. en Ankole, Ouganda de l'Ouest, par application de Dieldrine	W. R. WOOFF
25	Le foyer de trypanosomiase à *T. rhodesiense* au Burundi . . . . . . The focus of *T. rhodesiense* sleeping sickness in Burundi	L. VAN DEN BERGHE

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