study workshop on

PARASITE–VECTOR RELATIONSHIPS WITH PARTICULAR REFERENCE TO THE TSETSE-FLY

27 June 1 July 1973

SUMMARY OF PROCEEDINGS AND DISCUSSIONS

INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY (ICIPE) P.O. Box 30772 NAIROBI KENYA

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PREFACE

This conference, consisting of only 20 participants, was brought together as a result of the initiative of Dr John J. McKelvey, Jr., Associate Director of the Agricultural Sciences Division of the Rockefeler Foundation, as a kind of follow-up of an earlier Conference on Trypanosomiasis held at the Villa Serebelloni in May 1972. That particular conference was concerned mainly with parasitological and immunological aspects of this important African disease as it affects the vertebrate host.

We thought the biological relationships existing between the insect vector and the trypanosome parasite is a vital one, and needs to be thoroughly examined if we have to have a critical understanding of a disease such as trypanosomiasis.

We approached the problem by bringing together active workers in a variety of fields of direct interest to trypanosomiasis—tsetse ecologists and physiologists, epidemiologists, immunologists, and so on—who could bring to bear their own specialized knowledge on this transdisciplinary problem. The dialogue so established was most stimulating; and we are extremely grateful to the Rockefeller Foundation for sponsoring the meeting.

THOMAS R. ODHIAMBO,
Co-Chairman of the Conference.

Nairobi, Kenya,
9th November 1973.

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AGENDA

Wednesday, 27th June 1973

Evening

Welcoming Address		Dr. John J. McKelvey, Jr., Co-Chairman of the Conference
Address on the Villa Serebelloni	:	Dr William C. Olson, Director of the Villa

Adoption of the agenda and appointment of the rapporteurs.

Address on the Objectives of the Conference	:	Prof Thomas R. Odhiambo, Co-Chairman of the Conference

Thursday, 28th June 1973

Morning

"Tsetse Ecology, Epidemiology of Trypanosomiasis, and the Development of Trypanosomes."

1. The development of trypanosome infectivity in the setse fly: Dr L H Otieno.
2. Population ecology of tsetse flies: Dr L. C. Madubunyi.
3. Comparative study of the epidemiology of East Coast Fever: Prof W.I.M. McIntyre.

Thursday, 28th June 1973

Afternoon

4. The life-cycle of trypanosomes in the tsetse fly: Prof. R. Geigy.
5. The life history and morphology of trypanosomes in their insect vectors: Dr D.H. Molyneux.
6. Nucleic acids during the development of trypanosomes: Dr B.A. Newton.
7. Comparative study of the development of Anaplasma, Babesia, Theileria, and other tick-borne disease parasites, especially in their invertebrate hosts: Prof. Miodrag Ristic.

Evening

8. Film (16 mm., with sound) on the epidemiology of trypanosomiasis in livestock and game in the Serengeti Plains (Tanzania): Prof. R. Geigy.

Friday, 29th June 1973

Morning

"The Feeding Behaviour and Physiology of the Tsetse Fly."

9. The feeding physiology of tsetse flies and other blood-sucking arthropods: Prof. R. Galun.
10. Sensory-Physiology and behaviour of tsetse flies, especially in regard to feeding: Dr M.J. Rice.

11. The Functional relations of the tsetse salivary glands: Prof. Thomas R. Odhiambo.
12. Energy derived by the tsetse fly from its diet: Prof. E. Bursell.

Friday, 29th June 1973

Afternoon

"The Immunological Relations of Trypanosomes, especially in their Invertebrate Hosts."

13. Immunochemistry of trypanosomes: Dr A.R. Njogu.
14. Strain characterization of livestock and wild-game trypanosomes and the antigenic variation of *T.vivax:* Dr M.A.Q. Awan.
15. Experimental work with *T.rhodesiense* in mice and monkeys: Dr E.H. Sadun.
16. Comparative study of antigenic variation in protozoan parasites: Prof. E.A. Kabat.

Saturday, 30th June 1973

Morning

"Problems of Vectorial Capacity."

17. Vectorial capacity of tsetse flies: Dr Albert Challier.
18. Vector-host parasite interactions in the transmission of malaria: Dr R.A. Ward.

Discussion on the importance of the culture of invertebrate vector tissue: led by Dr R.A. Ward.

Discussion on proposals and Conference recommendations.

Evening

Discussion on proposals and Conference recommendations (concluded).
Closing Address: Dr John A. Pino.

DIGEST ON DISCUSSION

The Conference was opened on Wednesday, 27th June 1973, in the evening by Dr John J. McKelvey, on behalf of the Rockefeller Foundation and as Co-Chairman of the Conference. He gave a brief introduction on the history and reasons for the Conference, and then introduced the Chairman of the scientific sessions of the Conference, Professor Thomas R. Odhiambo. The latter set the goal of the Conference as one of exploring together, in a multi-disciplinary manner, the various approaches to a deeper understanding of the relationships between the trypanosomes and the tsetse fly, to discuss those novel and problematic aspects of this relationship, particularly as we are beginning to suspect that the vector may not be merely a neutral vehicle for the infective parasite.

Tsetse Ecology, Epidemiology, of Trypanosomiasis, and the Development of Trypanosomes

Dr Otieno opened the first session of the Conference on Thursday morning by making a presentation on "The development of trypanosome infectivity in the tsetse fly," especially as it relates to *T.brucei*.

He briefly described that the popularly accepted theory regarding the cycle of development of *brucei* trypanosomes has been questioned: for instance, a great deal of importance has been placed on the part played by the peritrophic membrane without enough supporting experimental evidence. Experimental evidence is lacking to show whether the peritrophic membrane is formed with each blood meal or whether once it is formed it stays for good. Furthermore, the significance of the lodgement of trypanosomes destined to reach the salivary gland needs to be re-examined.

Studies on the development of *T.brucei* in chick embryo cultured at a temperature of 39°C do indicate that bizarre forms of the parasites occur towards the peak of infection. Are these degenerate forms? Apparently, these forms are seen more often in a deficient immunologically reactive system (e.g. at the end of a chronic state or in irradiated host animals). He suggested that more studies were needed to evaluate the importance of these bizarre forms in relation to the life-circle of *T.brucei* trypanosomes.

Dr Otieno's paper was discussed by various participants; and the following points emerged:

1. Are the so-called bizarre forms of trypanosomes infective to insects?
2. The site of attachment of trypanosomes in the tsetse gut may be vital, for instance the question of whether or not electrical charges on the peritrophic membrane may be of importance for attachment should be investigated.
3. It is important to re-examine the development of the trypanosome in the insect vector, and find out the role of haemocoelic forms.
4. The surface coat of trypanosomes is apparently lost by the time the mid-gut is reached, and the coat is apparently restored when the trypanosomes subsequently reach the salivary glands.
5. The age of the tsetse fly may be a crucial factor in the success of establishment of infective trypanosomes in the fly.

The general conclusion was that a thorough re-examination of the developmental life-cycle of the *T.brucei* in the tsetse fly needs to be made, and to relate this to the physiological and biochemical events in the fly.

Dr Madubunyi ("Population ecology of tsetse flies") stressed the importance of several factors, that have hitherto been given only passing attention; in giving us an understanding of the population dynamics of *Glossina morsitans* in Zambia: pupal parasites (e.g. *Mutilla glossinae* and their effect on resident tsetse population, relation of the tsetse population to its preferred host (e.g. warthog) and the need for a simultaneous study of the population dynamics of host and insect, larviposition behaviour of pregnant flies and the selection of larviposition sites, and the types and distribution of resting sites. He noted that the problem of tsetse population regulation is likely to be complicated by the existence of sampling bias, and that further investigation of sampling methods should be carried out. Dr Madubunyi outlined a new, and more accurate, method for the assessment of physiological stress in tsetse populations based on the measurement of egg size.

In the course of the discussion that followed, the desirability was emphasized of reinvestigating the potentialities of pupal parasites as agents of tsetse control (Pino, Rice, Ward) and of exploring the possible effectiveness of microbia pathogens (Molyneux), though prospects were expressed concerning the prospect of success (Sudan, Bursell, Galun). The possible importance of mechanical transmission of trypanosomiasis by tabanids and other insects was discussed (Newton, Molyneux, McIntyre), but no consensus of opinion was reached on this issue.

The epidemiology of East Coast Fever was summarized by Professor McIntyre ("Comparative study of the epidemiology of East Coast Fever"), and differences from the trypanosomiasis situation were noted with particular reference to:

1. the permanence of infection in tick population;
2. the absence of wild life reservoires (buffaloes excepted); and
3. the stable, but patchy distribution of the disease, which is not clearly related to environmental factors, and which is confined to the East Coast and eastern part of Zaire. The reasons for this are not known

Control measures directed against the vector (dipping) and the parasite (immunization) were reviewed, and the possibility of deterioration in the control situation associated with the existence, or the development, of resistant strains, both of the vector and of the parasite, was noted.

During the discussion that followed, technicalities associated with the production of vaccine, and with the detection of possible resistant strains, were elaborated on (Ristric, Kabat, Molyneux, Sadun); and recent research which opens up the possibility of interferring with the mating of vectors by the use of synthetic pheromones and the interference of development by the use of hormones concerned with growth and moulting, was described (Galun).

Professor Geigy ("The life-cycle of trypanosomes in the tsetse fly") summarized the classical view of the developmental cycle of *T.brucei* in the insect vector, starting off with the stumpy form of the parasite in the blood meal. He noted that the number of trypanosomes taken up with a blood meal varies greatly, even when the blood is taken from a mammal sustaining heavy parasitaemia. He also noted that there is no evidence that trypanosomes can move directly from the tsetse mid-gut to the proventriculus and then to the salivary glands, and that it is possible that not all epimastigote forms reach the salivary glands.

Professor Geigy related new research findings that showed that the trypanosomes lose their external coat during their sojourn in the tsetse gut, and that it is possible that the parasite's Golgi apparatus might be responsible for the production of the coat surface in the salivary glands. Some time after the meta-

epimastigote forms have become established in the salivary glands, they detach from the latter and transform into metacyclic forms.

During the discussion that ensued, a question was asked as to whether the production of the coat substance during the salivary-gland stage was responsible for the detachment of the parasites from the glandular epithelium. Indeed, the whole problem of the initial loss of the external surface, its eventual reacquisition, and whether or not the two coats are identical immunologically was raised (Odhiambo). Finally, a lively dialogue (Otieno, Odhiambo, Molyneux, Njogu) revolved round the need to restudy the route of entry into the tsetse salivary glands of developing trypanosomes.

Dr Molyneux ("The life history and morphology of trypanosomes in their insect vectors") outlined the difficulties involved in the study of parasite-vector relationships using *Glossina* and *T.brucei* as study objects. Instead, he suggested that more attention might profitably be paid to (a) *T.Congolense* and *T.vivax*, in view of their greater veterinary importance, and perhaps (b) to other trypanosomes having vectors other than tsetse flies, such as *T.cruzi* or *T.rangelli* with their *Rhodnius* or *Triatoma* vectors respectively.

Such general problems as mixed infections, attachment mechanisms, and haemocoelic invasion could more easily be studied in *Rhodnius*, noting particularly the considerable background of information available concerning the physiology of *Rhodnius*.

Participants were generally agreed that such model systems would offer a useful adjunct to current studies on the tsetse/trypanosome interrelation. The mechanism and possible importance of attachment of trypanosomes to special cuticular or peritrophic membrane surfaces were discussed (Rice, Odhiambo).

Dr Newton ("Nucleic acids during the development of trypanosomes"), summarized recent work on kinetoplast DNA, a component which appears to be associated with the synthesis of mitochondral components in preparation for vector invasion; and indicated that the occurrence of isolated material in the form of small circular fragments should probably be seen as an artefact of isolation. He observed that marked differences in the kinetoplast DNA component had been found between different species of parasite, and that such differences might provide the basis of a field method for the identification of species, and perhaps of strains, based on DNA/RNA hybridization.

The problems of transcription mechanisms, and of the possible appearance of new DNA components during the pre-invasion amplification phase were raised (Odhiambo, Kabat) as was that of the possible involvement of kinetoplast DNA in antigenic variation, but no unequivocal answer could be given on the basis of available evidence.

Professor Ristic discussed the current knowledge of transmission, Prophylaxis, treatment trends, and the taxonomy of the various pathogens in his presentation on the "Comparative study of the development of Anaplasma, Babesia, Theileria, and other tick-borne disease parasites, especially in their invertebrate hosts". Discussion stressed the significance of transovarial maintenance of pathogens by ticks in contrast to the situation in insect vectors. Professor Galun suggested that further study of this problem could profitably be done on this problem. A great deal of interest was shown in the possibility of the separation and concentration of pure trypanosome populations with 'Sephadex', as well as in investigating the present indication that parasites undergoing schizogony within the mammalian host appear to take over the metabolic system of the host cells in a manner similar to that observed in virus infections.

The Feeding Behaviour and Physiology of the Tsetse Fly

The second theme of the Conference was opened on Friday by Professor Galun presenting a paper on "The feeding physiology of tsetse flies and other blood-sucking arthropods". She described studies on phagostimulants in a variety of blood-sucking invertebrates, and drew attention to the gradual specialization which appeared to have occurred in the course of evolution—from forms like the leech, which is stimulated to engorge by a variety of carbohydrates, and the ticks where the response to glutathione is synergized by glucose, to the insects in which the response becomes increasingly specific, narrowing from a general response to nucleotides in the primitive groups, through adenine nucleotides to stimulation by ATP in the most advanced forms. Under normal conditions the effective component of the blood meal, for such advanced forms, appears to be the nucleotides of the blood platelets, which are partially lysed during feeding, rather than the red blood cells themselves, which are not. Professor Galun put forward the possibility of developing a compound which would prevent engorgement by blocking the action of phagostimulants.

The problems associated with multiple feeding, and with the possible development of effective tsetse repellants, were discussed (Geigy, Rice, Bursell); and methods whereby platelet content could be reduced, thereby attenuating the feeding response, were mentioned (Ristic) though it was recognised that this could only be of theoretical interest. Possible interspecific differences in the density and disposition of mechano receptors on the proboscis of tsetse flies, associated with differences in the selection of preferred hosts (reptiles versus mammals) were mentioned (Molyneux).

Dr Rice ("Sensory-physiology and behaviour of tsetse flies, especially in regard to feeding") discussed the evolution of the blood-sucking habit in insects, as related to the evolutionary history of their hosts, and briefly outlined the main behaviour patterns of the tsetse flies in the light of current knowledge concerning the sensory control mechanisms associated with them, and the location of corresponding central nervous control centres. He then described the sense organs of the alimentary canal, and outlined the part that each played in the control of activities associated with feeding, noting particularly the sense organs situated at the tip of the proboscis, which he showed to be sensitive to saline (osmotic?) and to ATP stimulation. He noted the presence of secretory neurones associated with gut musculature and the gut epithelium, in some of which a discharge of neurosecretory material had been found to be associated with the act of feeding.

Differences between reptile and mammal feeders were further discussed (Molyneux), the possible existence of auditory sense organs was mentioned as worthy of investigation (Madubunyi), and the practical importance of work of the kind described was emphasized (Odhiambo).

In relation to the ultimate site of development of *T.brucei* parasites, and the subsequent injection of the fully developed parasites into a mammalian host, Professor Odhiambo ("The functional relations of the tsetse salivary glands") described recent work on the structure of the salivary glands of the tsetse flies, which were shown to comprise three distinct regions:

(a) a distal part characterised by a secretory epithelium, a fibrillar basement membrane and a substantial muscular investment, considered to be active both in the production of the primary secretory product (comprising granular and fibrillar components) and in supplying the mechanical

force requisite for salivary ejaculation;

(b) a middle part without muscle or fibrillar basement membrane, but with well-developed microvilli, thought to be involved in the further elaboration of the secretory material by resorption; and

(c) the salivary duct, with a thick cuticular lining and an epithellium inactive in secretion.

Professor Odhiambo demonstrated the presence of neurosecretary nerves associated with the muscles and the basement membrane of the distal region. Three types of inclusion were distinguished: a vesicular component presumed to be active in the control of muscle activity, and two granular components thought to be active in the control of secretory activity of the glands themselves. A hypothesis on the mechanisms for the control of salivary ejection was put forward.

The distribution of trypanosomes in different regions of the salivary gland, and their survival and development to the infective stage in that environment, was discussed, and it was agreed that attachment probably occurred in the middle region (Odhiambo, Molyneux, Geigy, Ward, Newton). The question whether trypanosomes should be regarded as parasites or symbionts was raised (McKelvey Molyneux) and the possible practical importance of a study of microbial symbionts (Rice) and of abortion (Madubunyi) was discussed at some length.

Professor Bursell reviewed the mechanisms by which energy is made available to the tsetse fly from proteins of the blood meal. He noted the importance of proline as a primary substrate for energy release, with particular reference to its high calorific value and ready mobilizability. The particular role of serine, glycine, arginine and histidine in the disposal of surplus nitrogen was described, as was the synthesis of lipids from the remaining amino acids. He noted that the utilization of lipid reserves as a source of energy does not follow conventional lines, and that it involves the coupling of lipid carbon to alanine, itself an oxidation product of proline, with subsequent synthesis of proline which serves as the ultimate substrate. Consequently, a cyclic system is involved, with lipid carbon as input, alanine as carbon carrier, and energy as the output. The relatively poor development of glycolytic enzymes was described; but it was thought possible that carbohydrate metabolism in specific organs (e.g. in Malpighian tubulus and the proventriculus) cou'd be important.

In discussion, the apparent importance of proline as a substrate for trypanosome metabolism was emphasized (Newton), as was the desirability of investigating the possible effect of trypanosome infection on tsetse metabolism (McKelvey), especially since it is known that trypanosomes possess large amounts of proline (Newton). It was observed, however, that proline is not a suitable source of food for tsetse flies.

The Immunological Relations of Trypanosomes, especially in their Invertebrate Hosts

The afternoon session on Friday dealt with the immunological relations of trypanosomes.

Dr Njogu ("Immunochemistry of trypanosomes") started the session by describing recent work on immunochemistry and fine structure of *T.brucei* subgroup trypanosomes. The work was aimed at obtaining a clearer picture of the nature of the variable antigens.

Enzymic iodination studies at EATRO, using radioactive $I,^{125}$ had revealed that two components of the antigen Complex were on the external surface of the trypanosome. Similar work with a different label (at Molteno Institute, Cambridge) had shown the presence of only one component on the surface. It was necessary to find the reason for this discrepancy. Since the antigens were presumed to be on the surface, work was started on the ultrastructure of the trypanosome external surface. Transmission electron microscopic studies revealed that the microtubules were interconnected by a lattice of fibres, while the flagellum was attached to the body of the trypanosome along a modified microtubule. The attachment started at the point of emergence from the flagellar pocket and continued all the way to the interior end of the parasite. A small fraction of the whole flagellum extended beyond the interior end of the body as a free flagellum. The flagellum was attached to the body by a row of desmosomes spaced at regular intervals along the modified microtubules. Freeze-fracture replicas indicated that each desmosomes consisted of a rose te of about 5 "microdesmosomes" arising on the flagellum and anchoring onto corresponding attachment points on the outer surface of the inner cells membrane. Dr Njogu stated that treatment with proteolytic enzymes unmarked reactive groups on the surface.

In the discussion that followed, it became clear that a great deal of interest centred on possible hypotheses and research needed to explain the mechanism of antigen variation in trypanosomes. If it was understood, it would probably be possible to block antigen variation and in consequence be able to prepare a practical vaccine for trypanosomiasis (McKelvey, Newton).

Dr Awan ("Strain characterization of livestock and wildgame trypanosomes and the antigenic variation of *T.vivax*") gave an account of the studies he is carrying out on the antigenic variation in *T.vivax* and on the isolation and characterization of trypanosome strains from game animals in Zambia. Two sheep were infected with a clone population of rodent-adapted strain of *T.vivax*. Both sheep were examined for peripheral parasitaemia daily for over 6 months. Each sheep developed a relapsing-type infection. Both sheep had 10-12 relapses up to 85-86 days after infection. At each relapse, mice were inoculated with blood from each sheep to isolate trypanosome antigen populations. Antisera were collected at the same time. Antigenically distinct population of trypanosomes were demonstrated by immune lysis and agglutination test. Altogether, three antigenic populations (T_7 T_{15} T_{21}) from one of the sheep were studied in more detail. The results indicated that exoantigens, bound antigens, and the corresponding antisera were produced in rats. Double diffusion of each of the exoantigens with homologous antibodies produced specific precipitin lines. Bound antigens showed the presence of a common antigen in addition to specific antigens.

Dr Awan also gave a progress report of his survey of game animals for trypanosomes. Four strain of *T.brucei* were found in hippo, but not *T.vivax* in any of 75 animals examined. Some species of antelopes had *T.vivax*. *T.brucei* was isolated from a lion.

In the discussion that followed, comment was made on the importance of the work on antigenic variation in *T.vivax*, which parallels the original work of Gray on *T.brucei*. It was pointed out, however, that caution should be exercised in using rodent-adapted *T.vivax* for experimental work. Finally, it was suggested that it might be important to re-examine the relation of the tissue form of *T.vivax* in relation to parasitaemia in the mammalian host.

Dr Sadun ("Experimental work with *T.rhodescience* in mice and monkeys") gave a progress report on this work since the last report he gave in May 1972 at Bellagio on the vaccination of monkeys, rats, and mice with irradiated *T.rhodescience* organisms. The aim of the recent work was to compare the protection induced by irradiated live trypanosomes to that produced by dead trypanosomes. The trypanosomes were exposed to gamma and neutron radiation. It was found that neutron irradiation inactivated trypanosome and produced protection at a lower dosage than with gamma irradiation. If various fractions of the parasites were used for protecting mice, it was found that the best "fraction" was that containing metabolic antigens (the buffer in which the trypanosomes were suspended), followed by the lyophilized fraction, followed by the particulate fraction. followed by the soluble fraction, These results indicated that living trypanosomes were not necessary for the induction of protection in mice. The haematological picture of experimental monkeys demonstrated that various parameters, such as enythrocyte count, packed cell volume, and haemoglobin concentration, increase in non immunized animals while they remained relatively constant for the immunized ones. Furthermore, a marked increase in gamma-globulin and a concommittant decrease in serum albumin was found in the non-immunized monkeys. Such monkeys developed a moderate hypoglycemia and, terminating, an increase in blood urea nitrogen. Transaminases (SGOT and SGOPT) were markedly elevated in the non-immunized animals. The pathologic picture showed that there was glomerular-nephrits; and the immune fluorescent tests showed the presence of IgM (and not IgG) antibody in the kidneys.

During the discussion of the paper, great interest was shown on the observation that protection had been provided by the "metabolic antigen". Professor McIntyre said that this appeared to be similar to the protection provided in *Trachinella*. Encouragement was given for studies leading to the characterization of these metabolic antigens. It was also felt that monkeys might prove an excellent mammalian model for immuno-pathological studies of trypanosomes.

Professor Kabat ended these discussions by outlining a possible approach to the study of the phenomenon of antigenic variation ("Comparative study of antigeniz variation in protozoan parasites"). If a *Paramecium* protozoan is treated with on antibody against its cillia the latter becomes immobilized. Later, however, it reacquires mobilization coincident with the appearance of a new antigen on the protozoan's external coat. This is really a case of antigenic modulation (rather than antigenic variation). It is imperative to explore the possibility that this phenomenon occurs in trypanosomes. On the question of immunization, Professor Kabat stated that there was a tendency—based on the success of such a system in virology and bacteriology—for scientists to study

such phenomena using a model host (usually a laboratory animal). Experience has shown that this approach has not succeeded in the study of immunization against parasitic diseases. It would probably be better for such studies to be concentrated in the animals in which disease is found, irrespective of the cost, while carrying on parallel investigations in laboratory animals.

Professor Kabat suggested several areas in which detailed information is needed in regard to trypanosomiasis immunology:

(a) A method for isolating enough antigens from different regions for comparative purposes; similarly, a bank is needed for reference sera from mammalian hosts;

(b) Since the varian antigens in trypanosomes appear to be glycoproteins, a biochemical study of the enzymes involved in the synthesis of these glycoproteins must be strengthened in order to give us a basis for the understanding of antigenic variation;

(c) Evidence so far suggests that the immune mechanism in trypanosomiasis is humoral; studies should, however, be mounted on the cell-mediated immunity to trypanosomes.

During the discussion that ensued, it was proposed that a thorough discussion on trypanosomiasis-resistant cattle should be launched at a suitable venue (Newton, McIntyre). What constitutes this resistance in immunochemical terms?

Problems of Vectorial Capacity

The Saturday session was opened by Dr Challier, who presented a paper on "Vectorial Capacity of tsetse flies". He said that Vectorial Capacity of tsetse flies depends on picking up the infection from the infected vertebrate host, development of the trypanosomes in the fly, and transmission of the trypanosomes to the next vertebrate host. There capacities are linked with: (a) endegenous factors of the tsetse flies (such as species, sex, age, physiology and host preference; (b) the trypanosome itself (that is, the infectivity of the trypanosomes, the various strains and forms, and the population); and (c) ecological factors (climate, presence of hosts, etc.). These factors are important, and studies have shown that temperature determines the duration of the life-cycle and the infection rate in flies. Thus, the infection rate of tsetse flies in the field was higher during the rainy season than during the dry season. In most *Glossina* species the infection rate for *T.vivax* and *T.congolense* is related to the proportion of blood meals taken from bovine animals. On the other hand, the infection rate of *T.rhodesience* and *T.gambiense* depends on the availability of suitable reservoir hosts. Dr Challier stressed the difficulties of evaluating the Vectorial capacity of tsetse flies: for instance (i) the genetic basis of vectorial capacity is not known; (ii) a revaluation of methods of trypanosome identification in tsetse flies is essential for a meaningful survey of the problem of vectorial capacity; (iii) it is not clear how we can best sample the epidemiologically significant tsetse population (as opposed to the whole tsetse population); and (iv) *T.gambiense* was usually diagnosed months after the original fly bite; by this time, the climatic conditions may have changed, and therefore the prevailing relationship between the human infection and the existing fly density may not be significant.

Great interest was aroused by the concept of vectorial capacity, and encouragement was given for further research in this area. It was suggested that a field technique for the rapid separation of trypanosomes in vertebrate hosts in circumstances of low infection rates was urgently needed.

Dr Ward ("Vector-host-parasite interactions in the transmission of malaria") reviewed the life-cycle of the malaria parasities of the genus *Plasmodium*. It was observed that although more than 500 different anopheline-mosquito species are known, only 30-40 are significant malaria vectors. Some of the more important reasons for this situation were discussed, and it was pointed out that: (a) the vector must be a species which feeds on man, partly or most of the time; (b) the degree of association between the human host and the vector should be intimate; (c) the adult mosquito should live sufficiently long for sporogonous development to occur; (d) the vector should occur during the same period of the year when the human host is infectious; and (e) the vector should be susceptible to malarial infection.

An analysis of mosquito susceptibility to plasmodial infection indicated that numerous, interesting factors are involved. Genetic variation is present at the specific level, within both the mosquito species and the local populations of mosquitoes. Studies on a single *Aedes aegypti* population indicated that resistance to infection by *Plasmodium* was largely due to a single gene. The relation of mosquito nutrition and the microbial flora of the midgut to plasmodial infection was described. Chemosterilants, such as TEPA, can interfere with both the host reproductive cycle and malarial oocyst development. Mosquito pathogens, such

as the microsporidan parasite *Nosema* prevent malarial transmission by occupying the normal oocyst substrate and utilizing host nutrienta to such an extent that plasmodial development is inhibited. Certain antimalarial drugs, such as primaquinine and pyremethamine, interfere with the development of the sporogonous cycle.

Various host factors affect the pattern of mosquito infectivity, the level of gametocycle maturity to malarial oocyst development in *P.falciparum* was analysed, the pattern of relapse and mosquito susceptibility in *vivax*-type infection was related to antigenic variation of the relapse strains. Parasite factors such as the geographic origin of the parasite strain and hybridization of parasite strains within the vector are also involved in mosquito susceptibility.

Recent studies on the response of malaria—infected mosquitoes on a flight mill were described. Malarial-infected mosquitoes had a shorter total flight range, reduced initial flights and had single flights of shorter duration than uninfected controls.

The application of these findings to studies on tsetse susceptibility were discussed. It was proposed that the genetic aspects of the susceptibility of *Glossina* to *T.congolense* in the mice be investigated due to the ease of working with this model in the laboratory.

Dr Ward followed this discussion by giving a review on "Trends in invertebrate cell culture". Over the past decade, there has been approximately a ten-fold increase in the number of workers in this area (from 6 to 60) the pioneering work of T. Grace led to the establishment of the first cell lines from the moth, *Antheridia*, and the mosquito, *Aedes aegypti*. These lines required haemolymph from the above moth as an essential component of the culture media. As techniques became better defined, cell lines were established from most of the major orders of insects. A significant advance was the substitution of foetal bovine serum for insect haemolymph. In recent years, many of the lines are being subjected to genetic analysis to determine the karyotypes of the cultured cells. This serves as an excellent marker to avoid contamination. At present, more than 50 cells lines are known from insects.

Various cell lines have been used to examine the interaction of the invertebrate phase of pathogens and a cell from the normal host species. Following the initial study of *T.vivax* in Glossina salivary gland explants more than 20 years ago by W. Trager, both I. Cunningham and I. Schneider have studied *T.brucei* in primary cell cultures from *Glossina* during the past year.

During the discussion that followed, Dr Newton pointed out that *T.brucei* had been grown on defined medium, could be transferred to new media, and still retain its vertebrate infectivity. It was concluded that further research should be conducted on tsetse cell culture and the behaviour of midgut forms in this environment, and that it be established whether they could eventually become metacyclic forms.

General discussion

Part of the morning session on Saturday, and the evening session was devoted to a general discussion on how the researches that had been described could lead to a new approach in the long-term control of trypanosomiasis—by immunization, by chemotheraphy, and by vector control. It was fully realised that any of these avenues need a great deal of investment in fundamental research if significant and rapid progress has to be made in this field.

Immunobiological aspects of the problem deserve special attention. Fundamental research should be undertaken with a view to the identification of antigenic trypanosome types, the result of which could serve as a basis for the development of effective vaccine. Concurrent to this line of research, it was suggested that the antigenic make-up of trypanosomes during their salivary-gland phase needs to be described fully, as a base-line for novel prophylactic procedures. In this respect, concentrated research effort should be focussed on the nature and development of metacyclic forms. It was emphasized that while empirical approach to immunization should be continued to get whatever practical immunization technology can be applied immediately to the trypanosomiasis problem, a more fundamental approach—requiring an understanding of the biochemistry and antigenicity—offered a better long-term solution to the lead that may emerge from the empirical approach. Fall-out of this fundamental approach might well include the development of better techniques for immuno-diagnosis of trypanosomiasis.

Trypanosomiasis control through chemotheraphy has shown that a few drugs have good possibilities. But prospects of future research in this field are meagre since the drug industry has lost interest in this field: it does not seem to offer much commercial returns for them.

Trypanosomiasis control through vector control contains several interesting possibilities. The sterile-male technique is one of the promising avenues; traps charged with attractants offer another avenue; but other approaches need to be explored so as to widen our arsenal for the control of tsetse population. Some of these novel approaches are: (a) to produce an antibody in the mammalian host which inhibits the activity of the anticoagulant in the tsetse salivary secretion; (b) study of the genetics of those tsetse populations that are susceptible to trypanosome infection; and (c) discovery of a specific blocking agent for neuromuscular or neurosecretory activity in tsetse flies. A great deal of controversial discussion went on around the subject of insecticidal control of tsetse flies; but it was the general feeling that deeper research should be carried out on these newer possibilities with the intention of controlling tsetse flies through integrated programmes.

Conclusion

The Conference was most productive. It brought together experts in a number of fields which do not normally work together in considering trypanosomiasis. The value of this confluence of ideas and expertise was most apparent. And it was the overwhelming view of the sponsors of this Conference and of the participants that meetings of these nature be convened at intervals to review progress in trypanosomiasis research and map out future strategies.

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