 Baluba 1

Month	ACCRA Mean Solar Maxima	Mean Terrestrial Minima	Mean Shade Maxima	Mean Shade Minima	Mean Rainfall Inches	Mean Relative Humidity per cent.	KUMASSI Mean Shade Maxima	Mean Shade Minima	Mean Rainfall Inches	Mean Relative Humidity per cent.
January	131·6	68·6	86·3	71·8	1·39	77·5	84·8	66·9	Nil	88
February	138·9	69·9	87·8	73·8	6·96	79·2	88·2	72·7	5·29	78
March	141·6	71·9	88·7	73·8	1·99	78·5	88·5	73·3	3·18	82
April	144·0	71·3	88·3	72·2	5·13	77·5	88·2	71·0	4·68	91
May	141·9	70·4	83·9	73·5	6·43	79·0	87·4	73·2	5·16	83
June	137·0	70·2	83·0	71·1	3·11	84·0	86·6	73·0	3·51	76
July	136·4	68·6	81·4	69·0	1·15	82·0	80·2	70·2	7·30	85
August	136·1	6·55	80·3	68·0	0·92	85·5	82·0	70·6	5·74	Wanting
September	139·5	68·3	83·7	70·9	1·25	83·0	83·0	70·7	11·79	85
October	141·5	68·5	84·0	71·8	2·95	85·0	84·2	70·8	7·33	83
November	141·6	69·0	86·9	73·2	0·93	80·0	86·6	71·5	1·93	75
December	135·4	68·5	87·2	73·8	0·72	77·0	85·2	68·9	2·16	72
Year	138·8	69·3	85·1	71·9	26·93	80·8	85·3	70·8	61·0	82
Number of Years included in Average	10	10	10	10	12	10	2	2	2	1

The readings of the sun thermometer run several degrees lower than in India, a fact that is explained by the veiling of the sun, during cloudless weather, by a peculiar haze, which is undoubtedly one of the factors of the comparative coolness of the climate, and is the more effective because it is most apparent during the dry season, when otherwise the power of the sun would have its full sway. It is said that, during a strong "harmattan" the darkening may rival that of a London fog, but if not an exaggeration, any-

thing approaching such a density is very rare. The presence of more or less haze, however, appears constant, during the Southern Solstice, so that during our few weeks' stay I doubt if on any occasion the sun shone with absolutely unobstructed power. The cause of this peculiar haziness is undoubtedly suspended mineral matter, brought by the wind from the great deserts of the hinterland, and extending to great heights, as the deposition of the sandy matter is often heavier far out at sea than on the shore.

It is nothing uncommon for literally bucketsful of sand to be swept up from the decks of a large steamer several times a day when steaming along the coast at this time of the year, and almost universally all varnished work is left with the wood almost stripped, and looking as if it had been sand-papered.

The dust that falls on shore appears to be much finer, and, though annoying, does not appear to occasion any irritation of the mucosa. The influence of this dust haze appears to extend at least as far south as the Congo.

The temperature records of the other G. Coast stations are too erratic to be worth preserving, though they confirm those of Accra, as far as they go. Those of the rain gauge are, however, given, as neglect of punctual examination of this instrument does not much affect monthly totals.

Considering the close proximity of Axim to Sekondi, and the absence of any great mountain masses to account for a local difference of rainfall amounting to more than double, the only possible conclusion seems to be that through some inadvertence the Axim rain-gauge must have been supplied with a measuring glass intended for an instrument of smaller diameter, or that there be some other source of fallacy. All the sets of record, however, show that the rainfall is somewhat capricious in alike its local and seasonal distribution. It will be observed that the two maxima of rainfall occur in June and October, but that, between the two, there is no sufficient period of dryness to affect the continuous breeding of mosquitoes. Thunderstorms appear to be rare, but small circular storms, of moderate severity, occur rather often. Though apparently of more frequent occurrence, they do not appear to approach in dimensions and severity the cyclonic disturbances met with in India and other parts of the tropical world.

The local observatories are, as already remarked, not too well installed, and, on the other hand, have a rather too ambitious equipment. It is doubtful if the information derivable from the solar and radiation thermometers is worth the extra trouble of observing them, except in observatories of the first grade, and these might be very well done away within the out stations, in which, on the other hand, the addition of self-recording aneroids might furnish a source of useful information. In any case, the appointment of a specialist to supervise the colonial weather records is a very obvious desideratum which it may be hoped will receive attention as funds become available.

A Comparison of the Conditions of European Life in India and West African Housing.

As a general rule, except from the point of view of the prophylaxis of malaria, Indian houses are well suited to the climate for which they are designed. They are nearly always constructed of materials available on the

spot, and, though not unfrequently in deplorable repair, owing to the conditions of tenure, are usually of rational original design. Their commonest fault is that of not being sufficiently elevated from the ground, but for the most part the rooms are lofty and well ventilated, and the materials chosen are of a character suitable to the exigencies of the climate of the part of the country in which they are situated. As a rule, however, Government buildings are not so well adapted to climatic needs, owing to the subjection of all other considerations to technical excellence in workmanship. Now, in an excessively hot climate like the Punjab, a thick wall of sun-dried brick forms a far better protection than a thinner one, even if built of the best burnt brick, laid in cement, and finished in the finest fashion ; while in the milder, but damp climate of Burmah, the more porous the walls are the better, and the old-fashioned European bungaloes, with most of the walls formed of split bamboo matting, always provided that adequate verandah protection be allowed, are much healthier and cooler than one formed of tongued and grooved planking or other impervious material. Fortunately for them, however, the number of Europeans who have to live in the cramped and uncomfortable structures, known as officers' quarters, is but small, as both civil and military officers have to hire accommodation for themselves, and have, indeed, to pay rent, even for a Government quarter.

The early Europeans, it must be remembered, had excellent models of the most suitable materials and type of building to construct in the dwellings of the native gentry, and even now some of the best houses available were built back in the time of the earlier European pioneers. In Bombay, and some of the other large towns, where there has been a tendency to make the houses look "so nice and English," the results have been deplorable to comfort, and doubtless to health.

In West Africa, on the other hand, the native does not rise above a beehive hut in architecture, and hence the European has had to evolve a tropical residence, like the German artist's camel, from his inner consciousness.

There appears to be an inexplicable reluctance to make use of any material obtainable on the spot, and the result is that structures formed of corrugated iron and wood, designed and framed up in England, form the bulk of the accommodation whether official or otherwise. Where more permanent materials have been adopted the result is usually simply an English house, with or without some apology for a verandah. There can be no doubt that the native hut, especially with a few obvious modifications, forms a far more comfortable residence than the best of these constructions, as, indeed, every West African officer who has tried both will tell you. It is, of course, quite possible to build a roof well suited to tropical climates even of corrugated iron, by constructing it double, with a wide and well-ventilated space between the two planes of metal, but, short of this, it is absolutely the most unsuitable material that can be chosen for the purpose. In the verandahs there is often the simple metal sheet, with the result that they are quite untenable as long as the sun is up, while in the rooms the metal may be, at best, supplemented with a layer of planking, separated from the iron by only the thickness of the joists, and absolutely unventilated between them. The only really scientifically constructed bungalow I met with was that of Mr. Dawe, at Obuassi, the roof of which was formed of shingles,

supplemented with wooden ceilings, separated from the roof by a ventilated loft. The district officers' quarter, at the same place, was, on the other hand, of the usual faulty plan, and though better placed, was, I found, no less than 7 degrees hotter on the same afternoon. Now a difference as large as this means ease and comfort in the one case, and discomfort and nervous prostration in the other. No one seems to have realised that the object of a verandah is not so much to afford open air accommodation as to protect the walls from the direct rays of the sun, and from the reach of any moderately driving shower. On this account, verandahs should be wide, and the lower border of the roof should reach fairly near the uoor level, say within six feet, in domestic architecture.

The verandahs of most of the houses examined were but 5 or 6 feet wide, with the eaves eight or nine feet from the floor, and the result was, that if one wished to rest during the heat of the day one often had to drag the bed about the room until one found a place to which the sun could not reach. Not infrequently, some of the walls have no verandah protection whatever, and, added to all this, the rooms are usually small, and so low-pitched that it would be impossible to swing an efficient punkah within them. The number of rooms, too, allotted to officers is very small in relation to the standard of comfort universal in the class of English society from which they are drawn, and this alone, quite apart from other considerations, makes it very difficult for a married officer to be accompanied by his wife, as, if she do so, she must resign herself to accommodation that would be considered barely adequate for the humblest of working couples by the least progressive of English municipalities. The one redeeming character of these structures is that they are usually well raised from the ground, being better in this respect than the majority of Anglo-Indian houses, out of Burmah and Assam.

The main reason of the unsuitability and inadequacy of the housing on the coast is the heavy cost involved in the importation of the whole of the materials. There is nothing whatever in the character of the materials available on the spot to prevent the building of houses exactly like those in universal use in Burmah and Assam, and there can be no doubt of their perfect suitability to the climate of West Africa.

As has been shown, there is very little difference between the climates, though that of Burmah is a trifle hotter, wetter, and subject to severer wind storms. In the hills in India we find that thatch resists wind better than corrugated iron, and though I am aware that thatch is said to have been tried in West Africa, and found wanting, the reason is very obvious, and lies in the fact that neither the natives nor the Europeans are expert at thatching. If, however, the negro be capable of being educated up to erecting tin houses, he can surely be taught how to thatch, besides which, there seems no reason why tiles should not be manufactured locally, and there are many other materials none of which could be as unsuitable as a mere film of metal, as a protection against a tropical sun. To conclude, there can be no doubt that in the matter of housing, the Anglo-Indian is infinitely better off than the white resident of West Africa.

Much as is the case with his house, the West African resident relies

mainly on importation for his provisions, and as a general rule, very little that appears on the table is produced in the country. In the case of meat, this is hard to be avoided, as there are but few localities where cattle can live, but even where they can do so, no attempt seems to be made to fit them for the table by feeding them. For the same reason, the poultry is extremely inferior. On the coast, the fish is excellent, but one is very apt to be offered tinned salmon, and in the same way, though there is abundance of excellent fruit, it is rare to find anything but Canadian apples put before one, and the writer has actually been regaled on preserved tomatoes in a place where a small but delicious variety of that fruit grew wild, within a few yards of the house. In the absence of cattle all dairy produce are necessarily imported, in the form of condensed milk and tinned butter, though it is difficult to see why, if aerated waters can be imported at as high a price as 7d. per bottle, milk should not be imported sterilized in bottles. Good milk and butter are so essential to all good cookery, that it is easy to see how the absence of the fresh articles must render catering difficult. The supply of fresh vegetables, too, is none too good. At the same time, it is probable that this absence of fresh milk and butter is one of the reasons of the absence of typhoid fever, as there is no doubt that, in India, these articles are among the commonest vehicles of the germs of that disease, as well as those of cholera and dysentery.

In India, on the other hand, except in Assam and Burmah, cattle flourish, and good meat can always be obtained, at any rate with a little management. For many years, those who desired a good and safe supply of milk were obliged to keep their own cattle, and have the butter made on the premises, but during the last few years European dairies have been springing up in all the principal centres, which supply excellent milk, fresh for local consumption, and sterilized for those at a distance, so that the necessity of running a private farmyard has become a thing of the past, except in a few isolated stations. In the same way, the old "mutton clubs," for the supply of grain fed mutton, are ceasing to be necessary, as the native butchers are beginning to realise that a good profit can be made by undertaking this business. The supply of vegetables is abundant and excellent, and that of fruit fair. There is great room for improvement, however, in the article of bread,which strange to say, is generally excellent on the West Coast of Africa, but on the whole, with good house-keeping, the European ought to be able to live almost as well in India as he can at home. Another great drawback of Africa, as compared with India, is that in the latter ice is abundant and cheap, whereas on the "Coast" a few pounds, begged from the weekly mail boat is regarded as a great treat, and inland it is quite unobtainable ; besides which, methods of cooling water and other beverages which are familiar to every Indian servant, seem to be understood neither by the African "boy" nor his employer. Now whatever may be the virtues of the fashionable hot water,there can be no doubt that the consumption of luke warm beverages is a great provocative of dyspepsia, and as a matter of fact, ice is for the European, almost a necessity of life in tropical countries.

The difficulty in the matter of aerated waters has already been alluded to, though it may be well to add that Indian experience shows that the size

of bottle best suited to the exigencies of the case is one that holds a pint.

A proper supply of good and well-cooked food is no mere luxury, but is one of the first necessities of health in any climate, and is doubly so in tropical countries, where the appetite and digestive functions are seldom at their best; and here, again, there can be no doubt that the Anglo-Indian is far better off, though it is undoubtedly the case that much might be done in Africa in the way of improving and utilizing local products, by the introduction of gardens, the setting up of factories for ice and aerated waters, and for the cold storage of imported meat. That the prolonged consumption of large quantities of tinned provisions is extremely deleterious is amply proved by the experience of every Arctic and Antarctic expedition, in spite of the most elaborate care in the selection and preparation of supplies of this sort. There can be no doubt that it is far better to put up with even inferior fresh provisions than to be enticed into consuming more than the irreducible minimum of tinned goods.

Domestic Service.

As is well known, Indian servants are very fairly efficient, but I must confess I was agreeably surprised by the African "boy." I have had far better servants in India than the "boy" who served me for a few weeks, but have also had more who were worse. Though said to be thievish, they seem generally cheerful and willing, and are far more generally useful than any single Indian servant, being quite willing to turn their hands to anything in a manner quite refreshing to one accustomed to have to consider whether or not the performance of any odd job would be against the caste prejudices of this or that attendant. They seemed very teachable, and as the degree of intelligence required to make a good servant need not be very high, it is probable that any superiority there may be in the average Indian servant is due to the fact that the latter has, for several generations, had the advantage of being trained by English ladies, for it is certain that the most domestic of men is a mere second class amateur as a housekeeper, compared with the average lady.

Condition of Life.

A very large proportion of Anglo-Indians are married, and are able to keep their families around them, at any rate until they become of school-going age, and it is needless to point out how greatly this conduces to health and comfort. If the British man has conquered India, it is his wife that has made his conquest habitable, and so much is this the case that it may be doubted if a bachelor is really much more comfortably placed there than if he served in West Africa, unless he be able to live at a mess or club. On the "Coast," ladies are only just beginning to make their appearance, and it is to their absence that the want of so many of the comforts and amenities of life is certainly due.

Conditions of Service, Accessibility of Health Resorts, etc.

In each of the countries compared, the European, especially if he belong to the public service, has much travelling to perform, but, though trying, at the present day in India, well appointed railways and good roads, available

for wheeled traffic, have done much to diminish the fatigues, while in the more backward parts of the country one can at least ride, and, if sick, can be moved in comparative comfort in a *duli*. Where there are no hotels, there are often Government rest houses, and, when on inspection off the main lines of road, the tenting allowances are sufficiently liberal to enable an officer to secure comfortable and safe accommodation at night.

The West African officer, on the other hand, still travels under practically the same conditions as those faced by the pioneers who have performed the work of exploration, the fatal character of which is too well known to require further comment. Putting aside the few miles of existing rail, there are no good roads, and as horses cannot live in the greater part of the country he must needs actually trudge the whole of the long marches, or at best ride in an uncomfortable contrivance known as a hammock. Moreover, the transport allowances are so scanty that the amount of the simplest necessities of civilised existence that he can take with him, if he have to travel or serve in the back country, is so small that it is impossible for him to enjoy even the most moderate degree of comfort, judged by European, or even Indian, standards.

Worse than all, owing to the entire absence of rest houses, to say nothing of hotels, it is actually the custom for officers to have to sleep in native huts, from which the inmates have been evicted for the night. Now, a native hut, in actual occupation, must necessarily swarm with infected mosquitoes, and it is difficult to understand how the greatest care and precaution can save the European who has to pass the night in such quarters from infection, to say nothing of the inconvenience to the native, at having to give over his house, which cannot fail to make the visits of officers unpopular, instead of, as they should be, a small event to be anticipated. The cost of erecting and maintaining huts of the ordinary native pattern along the more frequented routes would be trivial, and, if strictly reserved for European use, and placed at a safe distance from the huts of the village from which it is proposed to draw supplies and carriers, would do away with one very common method whereby officers become infected with malaria, to say nothing of tick fever. So obviously suicidal, indeed, is the custom of occupying native dwellings, that it ought to be absolutely prohibited, and the carrying of tents should be made compulsory. An 80lb. Kabul tent and the necessary camp-bed and furniture can easily be transported by three carriers, and there can be no doubt that the extra cost to Government for this small amount of transport would be repaid over and again in diminished invaliding. The substitution of some form of *duli* for the hammock is also very desirable. The Indian contrivance is so simple that it might be constructed easily enough by the ordinary workmen of an African village, and as one familiar with the difficulties of jungle paths, I have no hesitation in saying that the *duli* is far more suited to the country, for on account of its unwieldly length the hammock is as awkward a contrivance for the bearers to manœuvre through a narrow bush path, as it is uncomfortable for the unfortunate passenger. For the injured the hammock is absolutely dangerous, while for the sick it must be little short of torture, and added to this it would be difficult to elaborate a contrivance for transporting his fellow man, more

needlessly fatiguing to the bearers. The stalwart African bearers are of a physique that would enable them to walk off with an ordinary Indian kahar under each arm, and yet, it appears that they can only accomplish about two thirds of the distance that can be compassed by the latter, with his slightly heavier, but far more convenient, contrivance. The obvious fault of the hammock is that it cannot be rested on the ground without the passenger getting out, and this results in the bearers being kept in continuous muscular effort for too long a spell. The *duli*, on the other hand, can be rested on the ground without inconveniencing the passenger, and the kahar avails himself of this to frequently relax the strain on his muscles, and so is able to travel a greater distance, at quite as good an average speed as the African. A further advantage of the *duli* is that it does away with the necessity of carrying a camp bed, as on arrival, the pole can be instantly withdrawn and the frame carried into any tent or hut, while a large ground sheet, stretched over the pole *in situ*, converts it into a very passable shelter for the night. In the case of serious cases of illness, the advantage of it being unnecessary to disturb the patient on arrival at the end of a stage, is obvious. For persons in ordinary health, especially in close jungle paths, the ordinary "hill *dandi*" would probably be more convenient, and *a fortiori*, less awkward than the hammock.

The method of carrying the *duli* and *dandi* is practically the same as that of the African hammock.

At the present day in India, it is rare for anyone to be stationed more than two days' journey from a hill station, and there can be no doubt that this facility results in the saving of many lives every year, which would otherwise be sacrificed; but on the other hand, the dreaded Red Sea lies between us and Europe, so that the more thorough change to Europe is cut off during the hotter months of the year for really serious cases, though not a hot weather passes without a melancholy list of deaths of invalids in the Red Sea, who have either been imprudently sent home by their medical advisers, or more probably have started in defiance of his warnings.

In Africa, on the other hand, over immense tracts of country, no sites sufficiently elevated above the sea-level are to be found, and even where they exist, they have not as yet been utilised. Moreover, travelling is so slow that a hill-station could in any case serve but a limited area, and hence there is no possibility of change for anyone too ill to bear a long hammock journey. The accessibility of the Canary Islands is, however, a great counter-balancing advantage, for those stationed on the coast.

In the matter of leave rules, the West African officer is certainly the better off, as he can take about a third of his service on furlo' in England, while the Anglo-Indian is entitled only to one-fifth, in the case of the favoured covenanted civilian, and to much less under the varying rules of other branches of the public service. In both countries the conditions of private employ have a tendency to be modelled on those of the Government, but are naturally usually less liberal. In view, however, of the much more serious rates of sickness and death, to which he is exposed, it is certain that liberality in this matter to Europeans working in Africa is the

only policy consistent with real economy, as any retrograde step in this direction would certainly spell inefficiency in a very costly way.

Segregation.

The custom of the country in India ensures this, practically speaking, in as thorough a manner as is likely to be secured in any mixed community, and there can be little doubt that this is one of the main factors in bringing about the better standard of European health in India as compared with West Africa.

So much is this the case that when first the idea of the segregation of Europeans in Africa came before the professional public the writer was inclined to throw ridicule on the proposition, as he naturally read the word in its usual sense of absolute separation, because it never occurred to him that any Europeans would consent to live closely intermixed with the native population in the way that has grown to be the custom in Africa. In India the European quarter is commonly at least a quarter of a mile from the native town, and no one would dream of settling within the bounds of the latter, or even of taking shelter in a native house for the night, as, except perhaps in the case of heavy rain, everyone would prefer bivouacking in the open to running so heavy a risk.

A considerable number of natives are, of course, employed about the civil and military "lines" of an Indian station, but the great size of the ordinary Anglo-Indian's "compound" affords a very fair amount of segregation even from his own servants. There can be no doubt that caste prejudices on both sides have been the main cause of this satisfactory state of things.

Attitude of the Native Population with Respect to Sanitation.

Here, at least, the advantages are all on the side of Africa. No one who has not had to deal practically with the problems can form the least idea of the obstacles to every form of sanitary improvement which result from the tangled mass of caste prejudices and resulting habits of the natives of India. The mere fact that only outcasts can be employed on the most ordinary sanitary work, even of a domestic character, results in the universal fouling of the ground round every inhabited site, and converts the neighbourhood of every pool of water into a surface reeking with filth. For reasons of a similar kind, the Indian will only use a latrine under compulsion, and, in spite of his reputation for cleanliness, and usually fair niceness of person, he is very commonly unclean in his clothing, and generally so disagreeable a neighbour that it is in no way surprising that the Anglo-Indian has been led to "segregate" himself from him, without ever having heard of the system as a sanitary measure. Like, I suppose, most people who had never visited Africa, I had the idea that, from his lower grade of civilisation and intelligence, the West African native would be an even more unpromising subject for sanitary reform. Much to my surprise, however, I found myself among a population singularly cleanly alike in their persons and clothing, and far surpassing the lower class European in these respects. Added to

this, pit latrines are an indigenous institution in the native villages, and there is, therefore, no barrier of prejudice to prevent the West African making use of the better planned European appliance when it is provided for him.

Without entering into needless detail, it suffices to say that Sekondi is far cleaner than any Indian town of its size and resources with which I am acquainted, and that I further doubt if a whole battalion of police would keep the banks of the lagoons there as free from the offence as is the case, were its negro population exchanged for a similar number of caste-ridden Indians.

Only the Anglo-Indian does not live in close contiguity with the Indian, and the Anglo-African certainly does so with the negro, not because there is any particular difference in the necessities of the cases, but probably simply because the negro is not so obviously a bad neighbour as is the Indian.

Conclusions.

I have entered thus at some length into the details of European life in the two countries under consideration, because my visit, short as it was, has led to the conclusion that the greater sickliness of Africa is by no means as mysterious as it at first sight appears, and that it certainly is in no sense due to differences of climate, in the proper sense of the word ; as though the meteorological conditions of West Africa closely resemble those of parts of India, they are, all considered, not as trying as those of that country. It must be remembered that the European in the tropics is an exotic, and can only maintain a precarious existence by close attention to a number of alleviating details that need scarcely be taken into consideration in his native climate, and that this will continue to be the case however thorough the sanitary condition of his surroundings. For example, to take a simple illustration, no amount of sanitary reform, anti-malarial or general, will enable the European to venture abroad bare-headed in either India or Africa, and for the same class of reasons, the European cannot resist the influences of bad housing and bad feeding. To put it briefly, the main reasons for the better health of the Anglo-Indian are that he has made himself more comfortable in his novel surroundings, and that the very unsanitariness of the habits of Indian natives has led to the European's living quite apart from the native, and to his being less seriously affected as a sharer in the diseases of the native, in consequence.

Ask any Anglo-Indian to reside in a tin shanty, such as those I have described, in the middle, for example, of the Black-town of Madras, and to subsist mainly on a diet of tinned provisions, without either ice or punkahs, and I doubt if double pay and leave rules as liberal as those enjoyed by the Anglo-African will induce him to close with the offer, nor do I believe that he would be at all healthier than the Anglo-African were he to attempt to do so. Given close proximity, the European obviously cannot avoid sharing in the diseases of the native, and as he is living under exotic conditions, must necessarily suffer more seriously than the latter. The rates of native sickness and mortality are probably higher in India than in Africa, but the European, who there lives apart from the native, actually keeps freer from sickness than the natives themselves. If he lived in close proximity with

them it cannot be doubted that, like the European in Africa, he, too, would be more unhealthy than the natives around him, instead of less so.

These conclusions, assuming them to be correct, must be considered in the main satisfactory and hopeful, as it is obvious that apart from formal measures of sanitation the *desiderata* of comfortable living and segregation from the native depend mainly on individual initiative.

There remains the problem of Black-water fever, which, however, though it does not appear to affect him seriously, is also probably conveyed to the European by the native. The consideration already advanced constitutes, it is thought, a strong case against that disease being merely a severe form of malarial fever, and it is hoped may lead to renewed investigation and reconsideration of the problem, with a free mind as to the possibility of its having a distinct and specific causation.

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TRYPANOSOMES, TRYPANOSOMIASIS, AND SLEEPING SICKNESS

LIVERPOOL SCHOOL OF TROPICAL MEDICINE—MEMOIR XVI

REPORT

ON

TRYPANOSOMES, TRYPANOSOMIASIS, AND SLEEPING SICKNESS

BEING

AN EXPERIMENTAL INVESTIGATION INTO THEIR PATHOLOGY AND TREATMENT

BY

H. WOLFERSTAN THOMAS, M.D., McGILL

J. H. TODD MEMORIAL FELLOW

AND

A DESCRIPTION OF THE TISSUE CHANGES

BY

ANTON BREINL, M.U. DR. (*Prag.*)

J. W. GARRETT INTERNATIONAL FELLOW

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Dean of the School : Rubert Boyce, M.B., F.R.S.

The report is not so complete as wished for, but the immediate departure of Dr. Breinl and myself on an expedition to Brazil has compelled us to give our results in the manner here presented. We hope to fill in the omissions at an early date.

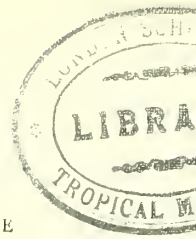
In conclusion I wish to acknowledge, on behalf of the research workers, the courtesy and interest which so many have evinced in the work. In particular Professors Boyce, Ross, Sherrington, and Moore, Dr. J. W. W. Stephens, Dr. J. Hill Abram, Dr. Lloyd Roberts, Professor Grünbaum, and Dr. Ernest Glynn, Dr. Lauder, Medical Health and Port Sanitary Officer, Southampton, his assistant, Dr. McCulloch, and the Matron of the Southampton Isolation Hospital.

I cannot close without expressing my indebtedness to Dr. H. E. Annett, who has done everything possible to aid the research department, to him and Professor Boyce the numerous worries and difficulties associated with the work have been left.

To the gentlemen on the committee and the Hon. Secretary of the School, who have endeavoured to meet all the requirements of the division, our thanks are tendered.

On behalf of the workers

H. W. THOMAS



CONTENTS

ERRATA AND ADDENDA

Page 4, line 12, *read* 'especially right inguinal and left cervical.'

Page 4, line 20, 'electrical reaction,' *read* 'electrical resistance.'

Page 7, Early history of Banja, Memoir XIII, L.S.T.M., p. 41.

Page 13, reference to Dutton and Todd, p. 97 of this volume.

Page 17, line 35, *read* 'small quantities of blood from an infected animal failed to produce infection.'

Page 30, Horse ; this is the animal referred to as 'Horse VI.'

Page 48, line 16, 'evidences,' *read* 'evidence.'

Page 49, line 4, 'Governors,' *read* 'Governors'.'

Page 49, line 8, 'Laver,' *read* 'Laveran.'

Page 49, line 10, 'potassium, and,' *read* 'potassium and.'

Page 50, line 14, 'Fowelri,' *read* 'Fowleri.'

Page 53, line 39, '889,' *read* '839.'

Page 59, line 4, 'are seen after,' *read* 'are seen but after.'

Page 60, line 20, 'subjects,' *read* 'substances.'

Page 62, line 24, '*P. rodigosus*,' *read* '*B. prodigiosus*.'

TRYPANOSOMES, TRYPANOSOMIASIS, AND 'SLEEPING SICKNESS'

BY

H. W. THOMAS

J. H. TODD MEMORIAL FELLOW

AND

ANTON BREINL

I. DESCRIPTION OF CASES OF SLEEPING SICKNESS IN MAN

OPPORTUNITY was afforded for study by the arrival in England of natives from the Congo Free State suffering from 'sleeping sickness' or 'Trypanosome Native Fever.' It is due to the courtesy of the authorities of the Congo Free State that facilities were given to the members of the Congo Expedition of our School to send to England selected cases of the disease. Special importance was placed on observations being made for a prolonged period in England where the change of climate, the regular life, and the general attention given to strengthening their systems might produce some effect on the disease. The cases observed were :—

Of 'Sleeping Sickness'	Of 'Trypanosomiasis' ('Trypanosome Fever')
Boyo	Mpangila
Kitambo	Disasi
Tomi	Banja
	One European Capt. 'S. B.'

It is not our purpose to discuss clinically whether 'Trypanosome Fever' cases are cases of early sleeping sickness or represent a distinct disease—this is left to the members of our Congo Expedition who have had opportunity of comparing several hundreds of cases. All the sleeping sickness cases died, the native 'Disasi' succumbed to pneumonia. Clinical histories of cases of trypanosomiasis in man have been so frequently recorded that a minutely detailed report of each case would serve no purpose.

Kitambo. Male, *aet.* 23. A native of Nyangwé.

History : Three years in Leopoldville. Was ten days in prison before coming to hospital. In prison felt ill and had pains all over the body. Could do no work, because of giddiness, headache, and weakness.

Condition. March 28, 1904 : Nutrition fair. Eyes heavy. Intelligence fair. Complains of frontal headache. Tremors of lips and hands. Gait steady. Skin moist. Glands, except epitrochlear, enlarged. Oedema of feet and shins.

Heart : Pulmonary second sound accentuated, rough and reduplicated ; apex in nipple line in sixth interspace.

Spleen enlarged.

Liver normal.

Lungs normal.

Nervous System : Knee jerks normal. Cremasteric reflex increased. Pupil reacts to accommodation and light. Tongue very unsteady.

April 15 : Sleeps a great deal in daytime. Unsteady gait. Very weak : cries for very little. Eyes sluggish to accommodation and light, pupils small. Tremor of legs when standing. Knee jerks and superficial reflexes increased. Nystagmus marked. During the voyage to England patient continued in about the same state. During the two days in Southampton he dozed most of the day. On arrival in Liverpool, May 24, he was able to walk to the cab. The man was placed in Dr. J. Hill Abram's Ward at the Royal Infirmary.

The following notes are taken from the hospital records by permission of Dr. J. Hill Abram :—

Present condition : Drowsiness and tremors. Lies on his back : not well nourished. Rash all over body. Sleeps a great deal. Slight glandular enlargement in left axilla and neck, and considerable in inguinal regions.

Alimentary System : Teeth good. Tongue tremulous and coated white. Abdomen slightly distended. No enlargement of liver, spleen, or stomach.

Circulatory System : Pulse frequent, soft. Apex beat just perceptible and palpable in fifth space, three inches from middle line. Sounds normal and no hypertrophy.

Respiratory System : Respiration frequent, tenderness and slight oedema over chest.

Urinary System : 2674 grammes, sp. gr. 1025, alkaline, deposit of phosphates, no sugar, no albumen, Ehrlich reaction negative.

Skin : Papular rash (scabies).

Nervous System : Very sleepy, general tremulousness. Pupils small. Knee jerk and plantar reflexes lively.

On June 3, 4, and 5, profuse sweating at night.

June 7 : Last three or four days seemed weaker. Cannot now feed himself nor walk. Cannot raise himself in bed. Urine and faeces passed involuntarily. Knee jerks present. No increase in drowsiness. Heart and lungs clear. Pulse, 108, softer. Occasional twitchings with considerable asthenic tremor in hands. Pupils small ; takes food readily, but has to be fed. Blood, haemoglobin, 65 per cent., red blood cells, 3,720,000, white blood cells, 7,812. Eosinophiles in comparison with the rest, not nearly so numerous as on May 25.

June 9 : Conjunctivae injected and muco-purulent discharge.

June 10, 2.50 to 3.15 a.m. : General convulsions, three fits, last fit continued for about ten minutes. Conjugate deviation of eyes and head to right. Both arms and legs worked, but right arm seemed to work more than the left, and movement was flexion and extension at elbow. Pulse 170.

CHART I

DISEASE.

Trypanosomiasis

Notes of Case.

Name Kitambo

Age

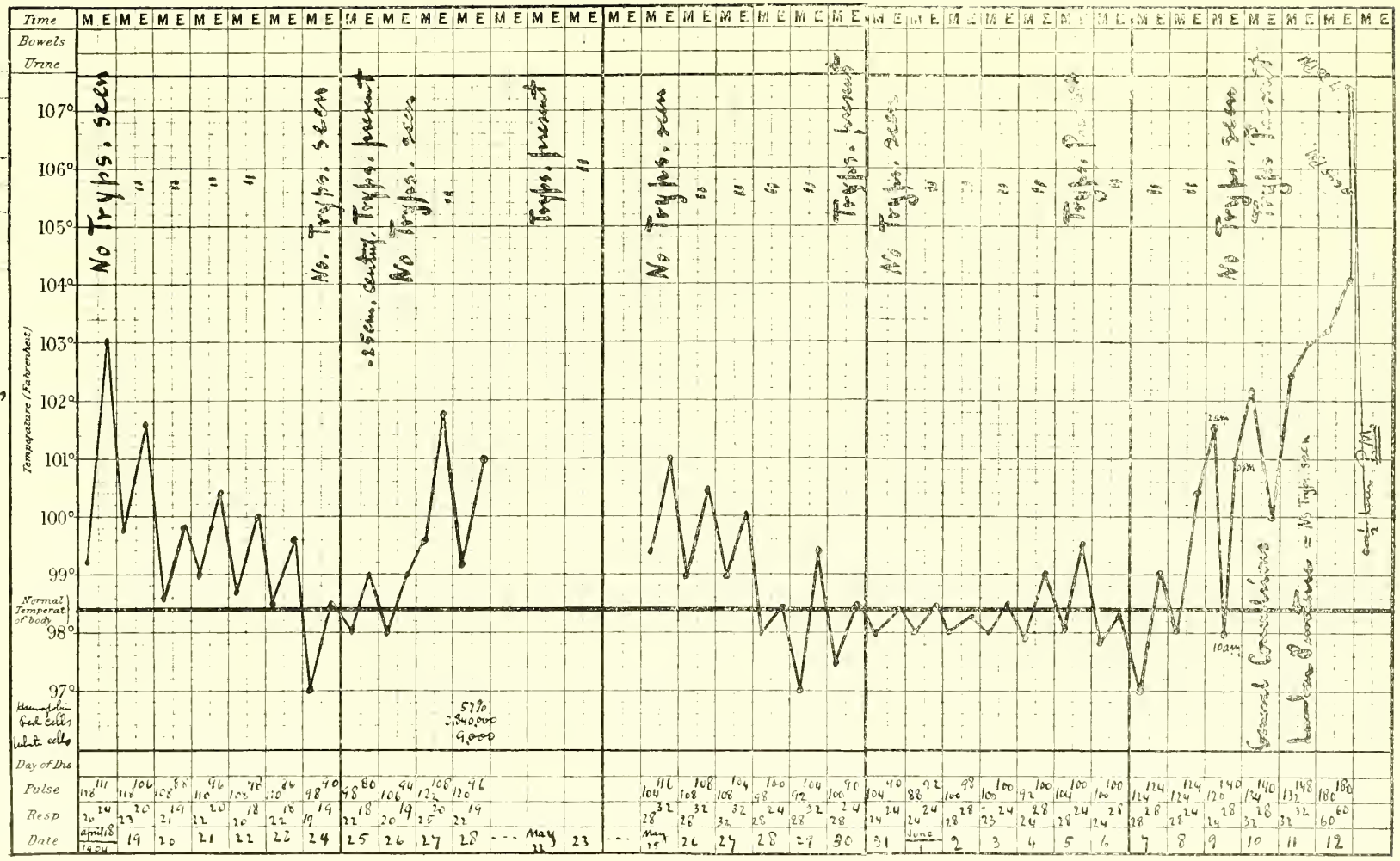
Diet

Case Book N°

Auto-agglutination of the Red corpuscles was constantly present.

Date of admission

Result



DISEASE.

Trypanosomiasis

Notes of Case.

Name { Tomi

Age.

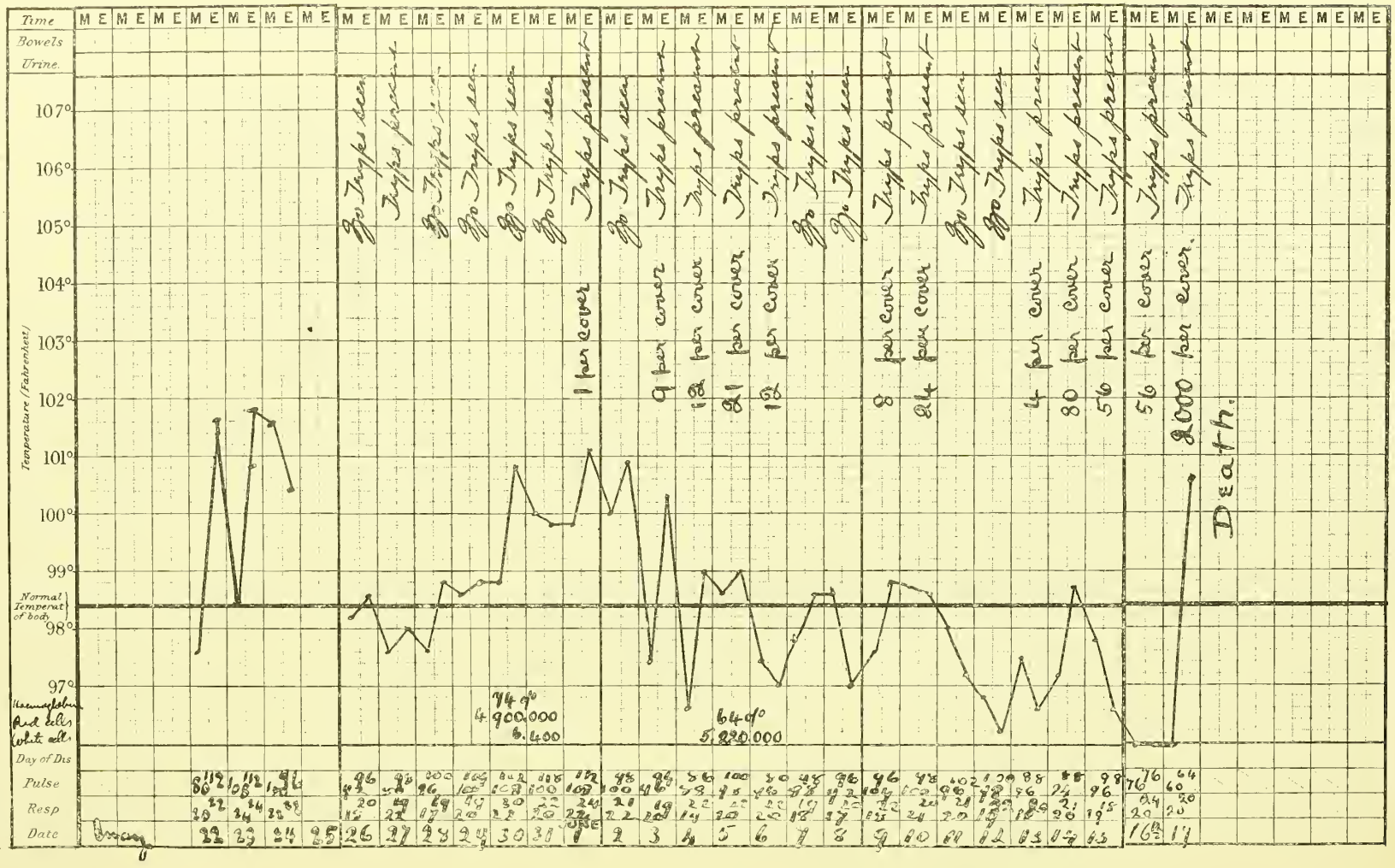
Diet

Case Book N°.

autoagglutination of Red cells constantly present.

Date of admission May 22/05

Result



7.30 p.m. : Unconscious most of day. At times seemed to hear when spoken to, but most of the time no response. Bowels moved from enema 2.30 p.m. and acted several times since. Croton oil, mid-day. Just had two general convulsions, lasting about three minutes each. Last fit, head and eyes first to left, afterwards to right. Arms and legs worked too. (Whilst writing a third fit came on). Conjunctivae much injected, good deal of muco-purulent discharge.

June 11, *midnight:* Semi-comatose during last twenty-four hours. No fits ; takes milk and whiskey when given. Corneal reflex present. Winced slightly when needle pierced skin for lumbar puncture to-day. No fluid ran out, a little drawn out by syringe, but mixed with blood. Profuse sweating on face, forehead, and neck. Past forty-eight hours, not much sweating on trunk. Pulse, 145, full, low tension. Respiration, 32 ; urine and faeces passed involuntarily.

June 12, 6.45 *p.m.* : Temperature getting higher all day and now 105·6. Pulse, 200, small volume, poor tension. Head and neck sweating still, trunk not. Respiration, 48 ; alae nasi working ; Cheyne-Stokes in type at times. Not been able to swallow fluid food to-day.

7.30 *p.m.* : T. 107·4 (thermometer seen).

7.45 *p.m.* : *In extremis;* face covered with cold perspiration. Corneal and knee jerk reflexes gone. T. 105·8. Heart beats 168 at apex, every third or fourth only reaching wrist. Respiration, 24, jerky (chin could be made to touch sternum).

7.55 : Respiration, 8 per min. Heart, 56. Violent last inspiration. Heart and respiration failed together. No P.M. rise of temperature, but fall (see chart).

For autopsy and morbid anatomy see Part II, Kitambo.

Tomi. *Aet* 18. Male.

He had been employed as cook to the Rev. Mr. Gordon at the Kinshasa Mission of the Baptist Missionary Society, Stanley Pool. As he was only seen a couple of times by the members of the Expedition, no detailed history is procurable, Mr. Gordon first noticed the boy to be careless, disinterested, and quarrelsome in December, 1903. In January, 1904, he complained of pains in one eye, which became inflamed. It improved for a time, but by the end of February both eyes were affected. This, coupled with the increasing irritableness and his being found sleeping at odd times in the cook house caused the missionaries to fear he had 'manimba.'[1] In April he was examined by Dr. Dutton, and parasites found.

On his arrival at Southampton, May 22, he was able to walk, but was extremely nervous and hysterical, laughing and crying without cause. Marked tremor of the upper and lower limbs when in a sitting or recumbent posture was observed. This condition was accentuated if the boy noticed anyone looking at him. On his arrival in Liverpool, May 24, he was very excited, and homesick, but became quiet on being given light work to do.

General Condition : A rather emaciated averaged-sized boy. Muscular development poor. Skin soft and clean—chiggers in both feet. Glandular enlargement general but not so marked as in other cases. Gait unsteady and very irregular, this is due to the chiggers. On standing still with the eyes closed he remains motionless for a few minutes and then starts to sway ; power over the knees suddenly appears to be lost and he will fall ; he can stand motionless if allowed to keep his eyes open, but after a while sways from weakness. At times, if seated, a violent tremor commences in the lower limbs, and heels rap the floor ; the knees, especially if the toes are resting on the floor, shake sideways and a little forwards. The hands and arms may remain quiet or start twitching spasmodically. The head usually moves sideways, the lips tremble, the eyelids twitch and the eyes roll. Plaintive sounds are emitted but no coherent words. These symptoms may cease after a few minutes, or a crying fit terminate the attack. If spoken to he can usually rise and appear quite well again, at other times, the trembling, etc., is not interrupted and the general vacant expression continues. Much is due to hysteria. If taken into a dark room and

1. A native name for 'sleeping sickness.'

his attention engaged, no swaying or falling occurs even if the heels are together. At night he will sit quite motionless without trembling. He is fanciful in his wishes and tastes, and very quarrelsome with his companions.

Respiratory system : Lungs normal ; expansion good.

Circulatory system : Heart, normal size and position. Slight accentuation of pulmonary sound. Pulse variable, often markedly irregular, quick, without tone, and soft, at other times of high tension with a slow but regular rhythm.

Digestive system : Teeth good ; faeces contain ova of *Ankylostoma duodenale.*

Liver not enlarged nor painful.

Spleen moderately enlarged, not painful on palpation.

Genito-urinary system : Urine normal, sp. gr. 1020. No albumen. No sugar.

ANALYSIS OF URINE MADE BY MR. EDIE, LABORATORY FOR BIO-CHEMISTRY

Date	c.c. Urine	Sp. Gr.	Reaction	Gram Total Nitrogen	Gram Urea	Gram Uric Acid	Gram NH_3	
June 17	325	1014	Neutral	3·14	5·82	·11	·05	Albumen and Sugar absent

Glandular system. All groups enlarged, especially right. Inguinal and left cervical freely moveable, not painful.

Aural and nasal systems normal.

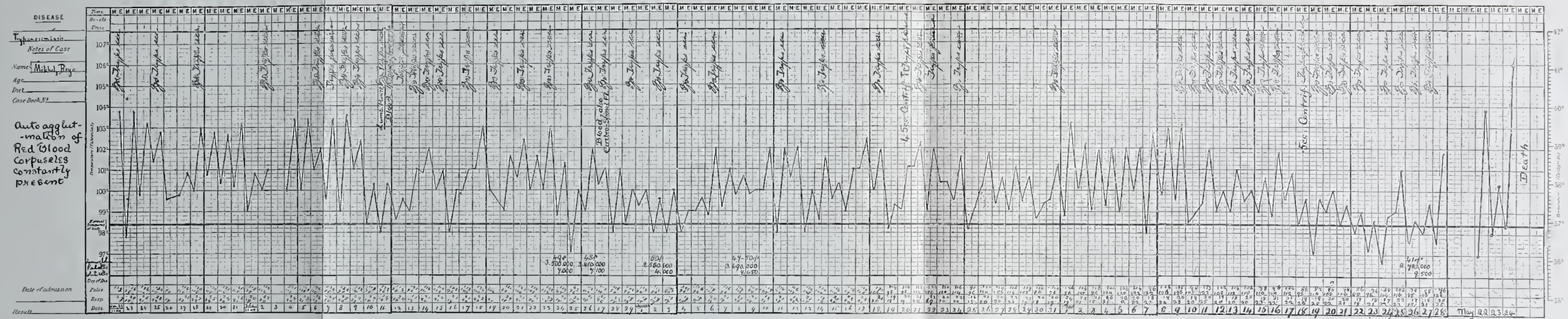
Pupil not dilated. Reaction to accommodation and light normal. Small opacity on cornea of left eye about 1 mm. in diameter.

Nervous system : Knee jerks increased. Cremasteric, epigastric, and anal reflexes not accentuated. Jaw clonus normal. Ankle clonus very accentuated at times.

Electrical reaction of skin normal. Some of the peripheral nerves reacted very slowly to the galvanic and faradaic currents. Some evidenced the degeneration reaction. The majority reacted normally.

From May 25 to June 11 the boy continued in the same state, seemed to gain in strength and became less excitable. The tremors became less accentuated and he learnt rapidly. At times he would become depressed and say he had 'manimba' and was going to die. *Sleep symptoms were not noticed.* During this period the parasites were always scanty, but appeared to be increasing on the 12th. On that day he finished his work and went to dinner ; at 2 p.m. the attendant came and said he could not wake him. He was found lying on his side with his knees drawn up, apparently asleep and perfectly motionless. On being shaken and shouted at he would open his eyes and utter a word and then relapse into the somnolent state. The temperature *per rectum* was 96·2° F. The cremasteric and epigastric reflexes normal, the anal much less. The knee jerk was much lessened. Respiration, 22, full and regular. Pulse, 100, soft, low tension, and irregular. After four hours he woke up and appeared to feel as usual, though somewhat dazed and unsteady in his gait. For the next two days he was kept in bed, but was always getting out and sitting on a chair. Parasites were increasing in numbers. The temperature continued sub-normal. On the 15th, 30 c.c. of cerebro-spinal fluid was drawn off by Dr. CHRISTY without difficulty, the fluid welling out when the needle entered the canal. The clear fluid, not contaminated with blood, contained trypanosomes, as many as five to a field. The blood at this time contained 700 to a cover, irregularly distributed, groups of two, four, and six being

CHART III



to face p. 5

seen. Part of the cerebro-spinal fluid was inoculated into a monkey, two rats, and a rabbit. The boy was kept in bed, though he said he felt well. The next day he was found in a semicomatose state. Temperature, 96·4° F.; respiration, 19; pulse, 108. Deviation of the eyes was noted. He was taken to the Royal Southern Hospital, and placed under the care of Dr. Lloyd Roberts. Until he died, on June 17, he remained in a somnolent state. At first nourishment in the form of soup could be given, but later total unconsciousness intervened. There was incontinence of urine and faeces. The temperature was sub-normal, but started to rise on the evening of the 16th, registering 100·6° F. at death. The reflexes were all decreased: sensations dulled. The pupils reacted to accommodation and light. The parasites continued to increase in numbers—a few hours before death two to three to a field were seen.

The differential blood counts made on two different days gave the following results :—

	June 11	June 16
Polymorphonuclear neutrophile ...	77·63	76·00
Large mononuclears	4·15	2·8
Lymphocytes	10·77	11·2
Eosinophiles	4·71	5·1
Transitional forms	2·14	4·2
Mast cells	0·60	0·7
	100·00	100·0
Trypanosomes	Absent	1 to 2 to a field

For autopsy, etc., see Part II, Tomi.

Boyo Mitchel. Male. *Aet.* 14. Coquilhatville.

First seen January 21, 1904. Came to Leopoldville two years ago. First felt ill coming down the Kasai river two or three months since, when he had headaches.

February 5: Thin and wasted. Gait slow, unsteady, and shuffling. Expression dull, apathetic, and vacant. Answers questions slowly and in a weak voice. Very drowsy.

Physical condition: Skin dry and scurfy. Glands enlarged. No oedema. Slight puffiness about eyes and puffy appearance of whole of face. Mouth full of sores.

Heart and Lungs normal.

Spleen not enlarged.

Liver apparently enlarged.

Knee jerks normal. Epigastric and cremasteric reflexes normal.

Faeces contain anchylostoma and ascarides.

Up to time of departure for England the boy remained in about the same state. Tremors being marked at times.

May 13: He walked with difficulty to the steamer. Temperature, 104 for two days; patient in state of collapse; could eat nothing. Urine and faeces passed in bed. After two days at sea he revived a little; the temperature fell and he was able to sit on deck. Mouth very foul. Later he was unable to get out of his bunk. Could only take soup and water if fed with a spoon

May 21 : Condition much worse. Emaciation very marked. Motions passed in bed. Bedsores over sacrum, others commencing on ankles, knees, and scapulae. Pulse extremely weak. Extremities cold. Gums ulcerated, teeth loose, mouth very foetid. Treatment of no avail.

May 22 : Taken to Isolation Hospital, Southampton. Condition after two days improved, but death took place May 24. Patient was lumbar-punctured six times, on three occasions parasites were present in the cerebro-spinal fluid. A rat injected with some of the fluid shortly after death, developed the disease in a mild form thirty-five days after inoculation.

For autopsy and morbid histology, see Part II, Boyo.

Mpangila. Male. *Aet.* 17.

Arrived in Liverpool, December 27, 1904. Parasites had been found in the blood in November, 1904, by Dr. Dutton.

Physical condition : A well-developed boy ; muscular development, fair. Thin, but not emaciated. Intelligence, above average. Easily tires ; no tendency to somnolence. Glandular enlargement general. Skin soft. No oedema. No eruptions on skin.

Respiratory system normal.

Circulatory system : Heart sounds, normal. Pulse, 96-104, soft and regular, at times becoming more rapid and irregular, without cause or relation to the temperature, rising to 110 and falling again to 96. This occurred when at rest or during work ; heavy labour did not appear to affect it.

Spleen : Slightly enlarged ; not tender on palpation.

Liver normal.

Genito-urinary system normal. Urine, sp. gr. 1018. No albumen ; no sugar ; chlorides ; no bilirubin. The following analyses were made by Mr. Edie, Laboratory of Bio-Chemistry :—

Date	c.c. Urine	Sp. Gr.	Reaction to Litmus	Grams Total Nitrogen	Grams Urea	Grams Uric Acid	Grams NH_3	Grams NaCl	Grams P_2O_5	Albumen	Sugar
July 9	1100	1015	Neutral	10·1	20·1	·46	·51	15·1	1·42	Absent	Absent
„ 14	940	1015	Acid	10·9	21·6	·56	·49	8·4	1·15		
„ 17	1160	1018	Alkaline	11·4	22·8	·69	·41	13·4	1·25		
„ 22	860	1024	Acid	12·3	24·8	·69	56	10·4	1·1		
Aug. 1	1300	1019	Acid	14·4	29·0	·71	·36	13·2	1·29		
„ 5	1700	1022	Acid	23·2	45·8	1·4	1·0	17	1·9		

Faeces contained ova of *Ankylostoma duodenale*.

Ocular, oral, and nasal systems normal.

Nervous system : Knee jerks normal. All superficial reflexes normal. No tremors. Pupils react to accommodation. Electrical reactions normal.

Sensations : Pain, temperature, taste, and smell normal.

Glands : All enlarged, especially cervical and inguinal regions. Easily palpable, freely moveable, firm, not painful ; some of cervical were as large as a hazel nut.

From December 27, 1904, to August 17, 1905, the boy was under observation. He was given work in the laboratory and animal houses. No evidences of the progressing of the disease could be determined. The boy grew taller and stronger and put on weight, learnt English rapidly, and his

intelligence improved. During the winter he contracted several colds, but was otherwise well. Rheumatic pains in the knee, shoulder, and wrist joints were complained of, but quickly disappeared. Somnolence was never observed. Easily excited and naturally irritable. Outbreaks of passion occurred at times; during such an outbreak he became frenzied and incapable of controlling himself. On one occasion severe frontal and occipital pains were complained of. At the same time it was noticed that the parasites were then starting to increase in number. The temperature also rose, but after two days the trypanosomes lessened in number, and coincident with this the headache disappeared and the temperature began to fall. A trial of subcutaneous injections of arsenic having proved too painful, liquor arsenicalis was given, but he quickly complained of headache and nausea. A varied treatment of Blaud's pill with arsenic, alternating with a nux vomica tonic and then liq. fowleri alone, was given. The blood counts still further improved; the parasites became scantier, and were often absent from the peripheral blood. The temperature, which at this time was irregular and sub-normal, became more regular, though very often sub-normal. Owing to his illtemper and homesickness he was sent back to the Congo in August, and the missionary authorities were given a supply of trypanroth tabloids for him. No news[1] has been received as to the boy's progress. Lumbar puncture was performed several times, but no parasites were found in the cerebro-spinal fluid, nor was such fluid infective to animals. The fluid possessed the normal characters; it did not agglutinate trypanosomes.

Blister fluid: Serum obtained from a small blister proved negative on two occasions. Mice inoculated with it, failed to become infected. Under the chapter headed Periodicity, tables shewing the presence and absence of the parasite in the blood are given. During the last twenty-two days, the parasites were hardly ever present, and were absent for the last eleven days before he left England, apparently due to the arsenical treatment.

	Differential Count	
	March 4	June 15
Polymorphonuclear neutrophile ...	53·98	61·81
Large mononuclears	6·78	5·63
Lymphocytes	10·32	8·36
Eosinophiles	25·69	20·91
Mast cells	0·68	0 48
Transitional	2·55	2·81
	100·00	100·00

Banja. Male. *Aet.* 26.

Under observation from January 18 to April 1, 1904. Arrived in England, May 22, 1904. The early history of the case has been recorded.

Physical condition: A large, strong, very obese man. Intelligence poor. Skin soft. General enlargement of the glands. Spleen just palpable, not tender. Liver not enlarged. Urine, sp. gr. 1018, no albumen, no sugar, no bilirubin.

1. On May 18, 1905, news was received that this patient was, in the middle of April last, in the last stage of Congo sleeping sickness. 'He was not always sleeping, but greatly emaciated, talks rubbish, and seems to have lost his reason.' Further details not yet to hand, July 20, 1905.—H. E. Annett. Aug. 30, 1905, patient dead.—J.L.T.

The following analyses were made by Mr. Edie, Laboratory for Bio-Chemistry :—

Date	Amount of Urine in c.c.	Sp. Gr.	Reaction to Litmus	Grams Total Nitrogen	Grams Urea	Grams Uric Acid	Grams NH_3	Grams NaCl	Grams P_2O_5	Albumen	Sugar
June 27	860	1014	Acid	10·5	21·3	·43	·26	3·62	...		
,, 30	1440	1019	Alkaline	17·9	34·1	1·29	·6	12·2	2·5		
July 13	1500	1017	Acid	14·2	28·2	·52	·76	19·6	1·5		
,, 16	1380	1011	Acid	9·5	19·0	·51	·4	11·3	1·4	Absent	Absent
,, 17	1480	1013	Neutral	12·6	25·3	·65	·34	10·8	1·35		
,, 22	1060	1020	Acid	10·5	21·4	·58	·34	11·1	1·2		
Aug. 1	2450	1014	Acid	20·7	41·9	1·2	·82	24·8	3·0		
,, 7	2800	1018	Acid	24·7	49·2	1·5	·97	23·1	3·4		

Nervous system : Knee jerks, cremasteric, epigastric, and anal reflexes normal. Electrical reactions normal, no tremors. Sensations, temperature, taste, and smell normal. No symptoms of drowsiness. No oedema, no skin eruption. The temperature ranged between 98° F. and 102·2° F., somewhat irregular ; sudden rises occurred which did not correspond with days on which an increased number of parasites was observed.

The pulse was as usual rapid, 94-108, once or twice rising without apparent cause to 128, usually regular and of good volume ; after climbing or work, irregular and dicrotic.

The man worked in the laboratory and animal houses. During the period of observation, from May 22 to August 15, no symptom other than the presence of parasites in the blood was noted. His appetite was inordinate and he grew extremely fat. No mental symptoms shewed themselves other than masturbation, which he had practised before arrival, and which he continued, despite all efforts to prevent it.

Lumbar puncture performed once by Dr Christy, and twice while under observation, never shewed any parasites in the cerebro-spinal fluid, nor were animals infected by injections of the fluid.

Lumbar puncture was performed three times in the Congo. Once trypanosomes were found but the notes record blood being mixed with the fluid at a period when eighty parasites to a cover were present in the peripheral blood.

Gland examination : One cervical gland was removed and examined on a day when there were only a few parasites in the blood. The juice did not contain so many trypanosomes as did the blood. Glands were punctured on two occasions. On a day when the blood was negative no trypanosomes could be found in the fluid withdrawn from the gland. On the other time both gland and blood preparations contained the same number of trypanosomes.

Blister fluid : The fluid contained no parasites, but successful infection of two mice out of four inoculated is recorded after a somewhat lengthened incubation period. This case was tried with injection of sodium arseniate, but as so much pain was complained of liquor arsenicalis was given by the mouth only to be discontinued and trypanroth gis. V in tabloid form substituted. To commence with one tabloid, later two tabloids a day were employed. No inconvenience appeared to be caused. The urine became pinkish twelve days after commencing treatment. A slight reddening of the conjunctivae appeared on the eighteenth day. The saliva appeared to have a faintly pinkish hue. The trypanosomes had

decreased in numbers apparently due to the action of the dye. As the man returned to the Congo on the twenty-second day of treatment the administration of the substance had to be discontinued. The temperature had fallen somewhat and the patient appeared to have experienced no ill effects from it. The patient arrived at Leopoldville in September, 1904, and was at once put to work. Through an unfortunate misunderstanding treatment was not continued. Four months later the patient entered hospital with evident signs of 'sleeping sickness,' and died there in June, 1905.—J. L. T.

Disasi. Male. *Aet.* 26. Lower Congo.

Arrived in Liverpool, December 27, 1903. He and his wife had been found to be suffering from trypanosomiasis. His wife died on the voyage to England.

Physical examination: A well-set-up sturdy man. Muscular development good, but inclined to obesity. Intelligence good; no signs of being drowsy. Skin soft. Few chiggers in feet. General glandular enlargement.

Respiratory system: normal.

Circulatory system: Heart sounds not accentuated. Pulse, 98-101, of high tension, but regular.

Spleen and Liver: Enlarged, not tender on palpation.

Genito-urinary system normal. Urine, sp. gr. 1020. No albumen; no sugar; chlorides; no bilirubin.

Faeces contained *Ankylostoma duodenale*.

Nervous system: Knee jerks normal. Epigastric, cremasteric reflexes not increased. Pupils reacted to accommodation and light.

Ocular, nasal, and aural systems normal.

The blood at first contained no trypanosomes—but *Filaria perstans*, often twenty-six to forty to a cover. Later, trypanosomes were very scanty; the examination was often negative. On January 26 an attack of malaria occurred, the temperature reacting to quinine.

February 6: Thirty trypanosomes to a cover preparation were found; these increased to eighty to a cover, and after four days decreased to seventy; they then disappeared, to reappear again later and remain more constantly present, though in small numbers. From that time until May 7, except for slight colds and an occasional headache, nothing abnormal was remarked. Glandular enlargement had not become more pronounced. On May 8 he had coryza, and complained of pain in the right ear; this increased in intensity. Examination revealed no mastoid tenderness or discharge from the ear. Signs of pneumonia appeared on May 10, and he was removed to Dr. J. Hill Abram's Ward in the Royal Infirmary. The disease progressed, and death occurred from pneumonia, May 14.

For autopsy and morbid histology, see Part II, Disasi.

Capt. S. B. *Aet.* 25. Italian.

Patient was employed in the Congo Free State River Service. As he was suffering from trypanosomiasis and was leaving for Europe, Dr. Dutton recommended him to come to Liverpool. No definite history as to the date of infection could be ascertained: he had had several attacks of malaria. The first symptoms of irregular temperature, not reacting to quinine, and slight oedema of the legs were noticed about the end of 1901. Trypanosomes were found in his blood by Dr. Broden, in September, 1903. Patient was under the care of Dr. Lloyd Roberts in the Royal Southern Hospital from May 31 to June 18. Our notes of the case record nothing abnormal in the physical examination other than a very slight enlargement of one or two inguinal and cervical glands. The liver and spleen were slightly enlarged and palpable. No tenderness was evinced. There was no purpuric eruption, nor could oedema of any part be found while he was in Liverpool. The parasites were very scanty in the blood. The temperature was of the usual type.

On *June* 5 an epileptiform attack occurred, he became unconscious for about ten minutes, the pupils were dilated, there was slight frothing at the mouth with loud stertorous breathing.

June 6 : Another slight convulsion, the pupils were very small. These fits were the first he had had. Before admission to the hospital he had been taking tabloids of some arsenic preparation. The drug was discontinued, but as he was not doing well liq. arsenicalis fowleri was prescribed. As he wished to go to his relations in Italy he left, promising to return but never did so.

Trypanosomes were only demonstrated in the peripheral circulation on June 4 ; a monkey, rabbit, guinea-pig, four rats, and four mice were inoculated with blood on a day when parasites were present and developed the infection.

Periodicity of the Parasite in Natives[1]

Below are given the number of parasites found in fresh coverslip preparations of blood from the peripheral circulation. These columns read downwards show the periodicity of the parasite.

Mpangila			Disasi			Banja	
3	1	4	0	0	0	46	10
2	4	1	0	30	0	32	0
1	2	0	0	50	0	14	0
0	4	0	0	70	0	300	0
2	3	3	0	0	0	4	0
1	6	2	0	0	0	16	1
0	1	5	6	2	2	40	0
0	2	2	0	0	2	40	0
1	0	1	2	4	0	0	0
0	3	2	0	2	0	0	0
2	6	7	0	0	0	8	0
0	8	5	0	2	0	2	0
0	6	2	0	2	0	0	0
0	0		0	0		16	0
0	2		0	0		16	0
1	2		0	4		4	0
0	4		0	2		0	0
1	0		0	0		0	0
0	0		0	0		12	0
0	2		0	0		0	1
1	2		0	2		0	90
0	0		0	0		0	21
0	0		0	2		0	2
5	2		0	0		48	0
0	6		0	0		6	0
0	8	rise of temp.	0	0		0	5
0	34	100·6	0	0		0	11
0	54	103·4	0	4		0	
0	12	101·4	0	0		3	
4	8		0	0		0	
5	2		0	0		1	
0	6		0	0		0	
0	14		0	0		7	

1. In a later report, periodicity of the parasite in animals as compared with other trypanosomes will be discussed.

PERIODICITY OF THE PARASITE IN NATIVES—*Continued*

	Mpangila	Disasi	Banja
Days present	113	22	26
Days absent	104	78	33
Longest period present—days ...	12	3	8
Longest period absent—days ...	7	25	13

These figures show that periodicity is very marked in some cases. The charts of these cases do not record any very marked rise of temperature coincident with an increase in the number of parasites. Mpangila had the parasites more constantly present but in smaller numbers than in the other two cases. From the periodicity columns the case of Disasi shows well the difficulty in making a diagnosis without daily examinations continued for a long period.

II. INOCULATION EXPERIMENTS WITH *TRYPANOSOMA GAMBIENSE*

Strains of Trypanosomes Derived from Man

In May, 1904, a preliminary note was published on a comparison of the animal reactions of various strains of trypanosomes derived from sleeping sickness cases in Uganda and the Congo Free State, strains from native fever cases from the same regions, with the original strains of trypanosomes brought back by Dutton and Todd from the Senegambia. Two Gambian strains were derived from natives suffering from the so-called 'Trypanosome or Native Fever'; the third was from a European who had died from trypanosomiasis. The strain obtained from the boy at Gunjur has been the principal Gambian strain employed, though the other two have been used to some extent. It is the 'Gunjur' strain which was sent to Professor Laveran, and also to Colonel David Bruce; from the latter we obtained Uganda sleeping sickness strain and Uganda native fever strain. From our expedition in the Congo Free State we have secured a large number of strains. We have been able to obtain fresh strains of trypanosomes from two cases of sleeping sickness and three native fever cases which had been sent from the Congo Free State to Liverpool for observation and treatment. We have also been able to procure a strain from a European who had returned from the Congo suffering from trypanosomiasis. It has been of great assistance to us to be able to compare strains derived from so many sources, and especially to be able to procure strains in their first passage through an animal and to compare them with those strains which had been through several hundreds of passages, and finally to compare them all with the parasite in the blood or cerebro-spinal fluid of a case suffering from the disease. In our report in the *Lancet*[1] we gave in detail the results of the inoculation of many of these different strains. Dutton and Todd have already published their results with the Gambian strains, also a short account of the animal reactions with various strains from the Congo Free State. Laveran and Mesnil have published their findings. Drs. Brumpt and Wurtz have recorded the results from strains derived from French Congo cases. All groups of observers have come to the conclusion that the trypanosomes found in cases of sleeping sickness are identical with the trypanosome found in the blood of a European, and described by Dutton in 1902. It would, therefore, seem unnecessary to reiterate any of the findings were it not for the publication by Plimmer of a comparison of the Gambian fever strain (Gunjur) and the Uganda sleeping sickness strain,

1. *Lancet*, May 14, 1904, p. 1337.

both obtained by him from Col. Bruce. A reply to the publication by Plimmer has already been made.[1] In correspondence with Professor Laveran, who has also experimented with both of the strains used by Mr. Plimmer, he writes that his results are equally opposed to Plimmer's. In order that Mr. Plimmer's findings may be compared with our results, a synopsis of his report is printed.

Further experiments and comparisons have been made with the various strains of trypanosomes. We have been able to successfully inoculate four baboons, one *Cynocephalus sphinx*, three *Cynocephalus babuin*, with strains of *T. gambiense*; these animals have all died. The baboon is certainly the most resistant animal with which we have experimented. From our baboon experiment, No. 747, a highly virulent strain has been recovered by passage through a rabbit.

Baboons (*Cynocephalus babuin*)

Experiment No. 709. Inoculated intraperitoneally July 24, from Rhesus, 672. Mixture contained two to six trypanosomes to a field. The monkey, 672, was a direct inoculation. On August 10 a temperature of 104·6° F. was registered; no parasites were seen. The animal lost weight, and the blood counts showed a diminished number of red corpuscles and of haemoglobin. No parasites were seen up to death, October 7. During the ten days preceding death the animal became very emaciated, and for the last thirty-six to forty-eight hours weakness was marked. Baboon lying down and almost unconscious. A rabbit inoculated with nearly ten c.c. of heart blood after a greatly prolonged incubation became infected. Up to date it has never shown parasites in large numbers.

Experiment No. 747. Inoculated intraperitoneally August 21, the temperature rose on the sixteenth day and after that date continued very irregular—104·2° F. being frequently registered. Death occurred on October 10, being hastened by a severe dysentery which commenced five days before. Parasites were first seen on October 1 and again on October 4, each occasion a high temperature was registered. In this case, as also in the other two, though the blood was so often or always negative still the characteristic clumping or autoagglutination[2] of the corpuscles was pronounced. This phenomenon occurred gradually but was easily determinable about the eleventh to fourteenth day after inoculation; it persisted to the end. At the autopsy, which was done immediately after death, a rabbit, 823, and two guinea-pigs were inoculated with large amounts of blood which was negative even when centrifuged. The rabbit littered on October 25, at the same time the temperature rose, the autoagglutination of the corpuscles was present but no parasites were seen. On November 1, 105·1° F. was registered; the next day, the twenty-third after inoculation, parasites were seen; these increased very rapidly so that thirty to forty to a field were present. From this rabbit

1. *Proc. Roy. Soc.*, 1905. 2. Dutton and Todd.

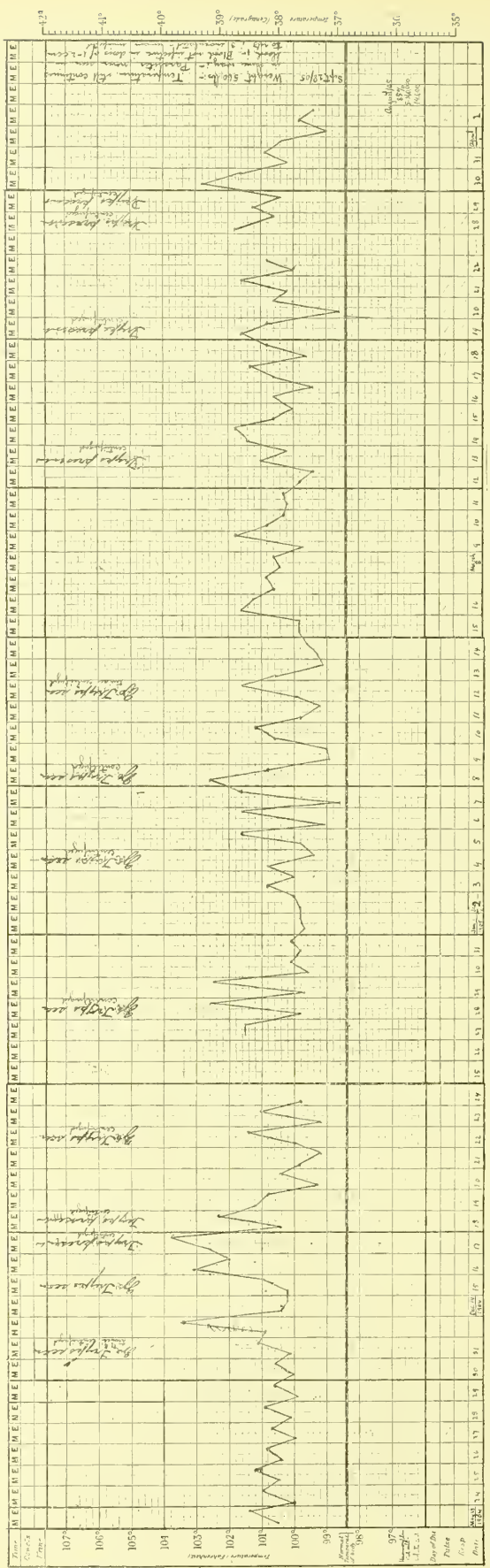
a series of animals have been inoculated. The incubation period and duration of the disease is much less than with the ordinary strain of parasite. One of the guinea-pigs has since shown parasites in its blood. Whether the parasite from this guinea-pig is as virulent as that from the rabbit is not as yet determined. The animals died four-and-a-quarter weeks after the appearance of parasites, there being over one hundred to a field at death. Further experiments in progress.

Experiment No. 890. Inoculated intraperitoneally with 9·0 c.c. pure blood from rabbit 877. Mixture contained one parasite to a field. This rabbit was inoculated in the third passage from rabbit 823 (see above). On the tenth day the temperature rose to 103·4° F. No parasites were seen. Two days later a trypanosome was discovered. From that time until the baboon's death on the forty-first day the organisms were present at nearly every examination. Death took place suddenly. A pup inoculated with heart blood from this baboon developed the disease after a prolonged incubation, the parasites were present in only very small numbers up to its death.

All three baboons showed a rise of temperature to 103°-104° F., and following on this an irregular temperature which persisted, usually becoming lower and often being sub-normal before death. Parasites were found in the blood of only two of the baboons despite repeated centrifuging of the blood, but though the parasites were hardly ever present, and then only one to two to a cover, still animals inoculated with large amounts of their blood have developed the disease. Loss of weight and anaemia, though gradual, was present in all three cases. The autopsies, especially of 747 and 709, showed an enlarged and firm spleen, slight enlargement of the lymphatic glands. Dr. Breinl has included the brains, cords, and organs of these animals in his report on the histo-pathology.

The strain derived from baboon 747 and passed through rabbit 823 has been inoculated into a great number of animals. In all the incubation period has been greatly shortened. Rabbits very often show parasites in their blood in two to four-and-a-half days, and death may occur as early as the fifth to eleventh days after inoculation. In many cases the parasites augment from day to day until there will be one hundred and fifty to two hundred or more to a field. The animal will very often suddenly die. The number of trypanosomes will frequently decrease, they usually commence to increase in numbers again in three to eight days, and continue to do so until the blood actually swarms. A very severe anaemia occurs, loss of weight is marked. Coma may develop a few hours before death or the animal may die while in the act of eating and apparently quite strong. The temperature is usually very high, the incubation rise being often to 104°-106° F. It usually continues high, hardly falling at all in the acute fatal cases. The *post-mortem* of these acute cases shows a multitude of parasites in every organ and in the blood and serous exudates, acute swelling of the spleen, enlargement of the glands—some of these may be haemorrhagic.

CHART IV



to face p 15

Rats, mice, dogs, cats, guinea-pigs, and monkeys develop the disease in equally short incubation periods. Death occurs early: in the dog in nine days; cats, twenty days; rats, twelve to eighteen days; mice, twelve to sixteen days; monkeys, ten to twenty-four days. Guinea-pigs may die in twelve days to three weeks after the appearance of the parasites in the peripheral blood. The guinea-pig may resist, and after an incubation of three to five weeks acquire a more chronic form of the disease.

Monkeys

Various species have been used, *Macacus rhesus*, *Cercopithecus callithricus*, *Cercopithecus ruber*, *Pithecia satanas* (*Jew* and *Sooty* monkeys). All react to inoculation with almost the same sensitiveness. The *Sooty* appears to be somewhat less so, the parasites are rarely seen but autoagglutination of the corpuscles, anaemia, fever, and loss of weight occur as in the others. Monkeys, though reacting so well and the parasites often showing in large numbers, are, unfortunately, extremely liable to digestive disturbances, their vitality is not great and they quickly succumb to the complication. As they usually harbour large numbers of intestinal parasites the blood counts cannot be absolutely depended on. The monkeys infected with sleeping sickness strains have not shown any more symptoms of 'sleep' than others inoculated with 'Gunjur,' etc., strains. Monkeys infected with *T. dimorphon* and non-infected ones dying from dysentery, etc., have sometimes exhibited even more pronounced symptoms.

It is of interest to record the progress of the disease in large animals infected for a long time.

Horse VII

Experiment No. 87. Inoculated by Dutton and Todd, February 14, 1903. Strain 'Mr. Q' (European, Senegambia). Parasites appeared March 16. Up to May, when it was sent to England, the organisms were fairly constant in the peripheral blood. From August 31, 1903, the blood was negative to microscopical examination, but still infective if inoculated in large amounts. On numerous occasions, the blood was centrifuged, but no parasites could be found. The blood when inoculated in amounts of 0·5 c.c. into rats and mice, formerly proved infective, such a quantity is at present of no use; 2·5 to 3·0 c.c. or more is now needed to produce an infection. Moreover, it is necessary to use more than one animal, as the animal may only acquire a mild chronic infection with very few parasites to be seen in its blood. The horse, shortly after the infection, lost flesh but improved after arrival in England. It is now in excellent condition, weight, 510 pounds, no oedema. The blood count: reds, 5,600,000; whites, 17,600; haemoglobin, ninety per cent. Urine normal. The autoagglutination of the blood corpuscles is still present. Its temperature has been recorded for nearly the whole period (see chart); glandular enlargement has not been noticed since the animal arrived in England.

Donkeys

Two were inoculated, one with 'Gunjur' strain (*Lancet*, May 14, 1904), the other with Uganda sleeping sickness strain, between February and March, 1904. These animals became infected, and showed the parasites in their blood. The parasites have always been extremely scanty in numbers, even after centrifuging the blood. The occasions on which the blood examination has been negative have gradually become more frequent, but they are still occasionally seen. The autoagglutination of the corpuscles is marked. Few symptoms have been observed. For about two months after infection, the animals appeared to be losing flesh and were less lively, they have now regained their normal weight. Anaemia has been present from the commencement but it is not marked. The temperature has continued irregular, varying between 98·6° and 100·4° F., occasionally it rises to 101·8° to 102·4° F. about every ten to fourteen days or so. This rise continues for about forty-eight hours, when the temperature drops to normal or sub-normal. The urine is negative. Glandular enlargement is present in both these animals, it was noticed about a month after infection, and still continues, though much less marked. The blood is infective to rats in amounts of 0·5 to 1·0 c.c., formerly a few drops of blood were sufficient.

Cow

This animal was inoculated, April 28, 1904, the temperature rose to 103·6° F. eight days later, but no parasites were seen until May 17. A rat inoculated on the day of the rise, developed the disease. For the first month no symptoms were noted, the animal then began to lose flesh, and became quite thin, but in July it commenced to put on weight. The yield of milk decreased, the parasites were extremely scanty, and have been present in less numbers and at longer intervals than in the case of the donkeys and the horse. Anaemia was not marked. The blood count at present is, reds, 6,220,000; whites, 16,200; haemoglobin, eighty-five per cent. Autoagglutination of the corpuscles has been present from May 20—it still persists, but is not so marked. The temperature has shown the same irregularity, rising gradually but irregularly for ten days to remain high for about one-and-a-half to three days, and then commencing to fall. Glandular enlargement was present up to August, 1904. The coat which had become rough and dull looking is now smooth and glossy. The blood is still infective though larger amounts are now necessary.

A peculiar condition has been noted in the milk. About two-and-a-quarter months after infection, it was noticed that the milk was very poor in fat, and watery. Dogs fed with it developed in about twenty-four to thirty-six hours a very profuse diarrhoea, which continued so long as the milk was given. Pasteurising or autoclaving at 120° C. appeared to make no difference, as pups and kittens developed diarrhoea in two to four days. Bacteriologically, the milk has been proved to be free from pyogenic organisms. Guinea-pigs inoculated with it have not developed any lesions.

Chemical analyses have failed to detect any changes other than a low percentage of fat. Tried on human beings, nothing is noted other than a tendency to cause biliousness and diarrhoea. The possibility of antibodies being formed in the milk has been recognised, but no proof has been established.

Sheep

A wether, Experiment No. 560, became infected ten days after inoculation. On the twenty-seventh day one parasite to a field was noted. The numbers gradually decreased, and five months later, the animal appeared to have recovered. Blood in amounts of ten to forty c.c. was non-infective. Slight anaemia was present for a time. Autoagglutination was fairly well marked until the end of the third month.

Goats

The animals inoculated with the various strains have all died. One, No. 480, inoculated with Uganda sleeping sickness died on the fifty-eighth day. The anaemia was somewhat marked. The parasites were present fairly constantly, usually four to twenty-four to a cover preparation being seen. Autoagglutination of the corpuscles was not very marked. The temperature remained irregular. Loss of weight was the only symptom noted. The *post-mortem*, twelve hours after death, showed a slightly enlarged spleen and glands, none of them haemorrhagic. Decomposition was advanced.

Dogs

These animals, though easily susceptible to inoculation, survive for many months. Laveran records one dog still living, six months after infection. A bitch, No. 738, inoculated with 'Gunjur' strain lived for over nine months. The parasites were at first hard to find, but for the last three months became more and more numerous. For the first two months emaciation was not marked; a progressive decline then became evident, and this continued up to death. Towards the end a profuse discharge from the eyes occurred; the conjunctivae were inflamed and infiltrated.

The blood count of this animal is interesting (p. 18).

Some dogs have died in three weeks, others have lived for varying periods up to nine months. A bitch, Exp. 45, brought back by Dutton and Todd, recovered from the disease. This animal had been inoculated when a puppy; it developed a chronic form of the disease and at the end of four months no parasites could be found in its blood. Autoagglutination of the corpuscles gradually became less marked, and ten months after inoculation the blood appeared perfectly normal. In amounts of 2·0 to 10·0 c.c. it was non-infective. Reinoculation of small quantities of blood failed to produce infection, although a control dog developed the disease.

Experiment 738

Day of Disease	Red Corpuscles	Leucocytes	Haemo-globin	Parasites
4th	5,240,000	12,000	85	8 to cover
5th	6,500,000	12,600	86	40
6th	5,430,000	15,800	75	228
7th	5,610,000	17,000	65	400
8th	5,850,000	18,600	64	2400
9th	5,540,000	13,600	79	24
15th	4,270,000	18,400	60	56
58th	5,260,000	10,000	69	28
69th	7,820,000	11,800	92	2
166th	4,310,000	14,000	70	680

It appeared to be immune. Its serum did not possess protective properties. Infection and progress of the disease occurred in all animals treated with this serum. Its agglutinative properties were infinitesimal. Pups born of this bitch were as susceptible to the various strains as the control ones. The temperature varies. Usually, in the long continued cases, exacerbations of fever with apyrexial periods of eight to ten days occur ; sometimes the fever is high and irregular during the whole course of the disease. A subnormal temperature is usually recorded before death. One animal developed fits eight days before death, and unconsciousness became marked eighteen hours before the end. Owing to absence from the laboratory no *post-mortem* was done. Anaemia may or may not be pronounced. Autoagglutination is usually pronounced.

Pups are very susceptible and usually show large numbers of parasites in their blood. Periodicity is sometimes very pronounced, the parasites often diminishing from ten to forty to a field to only a few in a coverslip preparation. This may occur in the course of a few hours.

Cats

As shown by us, these animals are susceptible to inoculation. The disease runs a course of three to ten weeks ; a few have lived for a longer period, six-and-a-quarter months. Parasites are generally hard to find, but can be usually found if properly looked for. The temperature is irregular, sometimes passing 104·5 F., apyrexial intervals usually occur. It is often subnormal about twenty-four hours before death and there may be a steady and regular fall for some days preceding death.

Anaemia may be a prominent feature. The blood count of one cat was, reds, 8,600,000; whites, 6,000; haemoglobin, seventy-eight per cent.; on the twenty-ninth day of infection, reds, 3,470,000; whites, 14,400; haemoglobin, thirty-eight per cent. Its weight had decreased three hundred and twenty grammes during this time. The parasites in this animal were numerous, at times three to a field were seen. Auto-agglutination is a noticeable feature both in the acute and the chronic types of the disease.

Kittens are very susceptible, the parasites appearing early and generally persisting in large numbers until death. The anaemia is severe. Loss of weight and stoppage of growth occurs. A high temperature is noticed. The duration of the disease is from three to seven weeks. Purulent conjunctivitis is common.

Rabbits

The incubation period has not varied markedly; from the figures given in the preliminary report five to fifteen days. Some of the rabbits have lived one hundred and fifty to two hundred and seventy-three days; these have had a chronic, mild infection. Average duration fifty to one hundred and twenty-eight days.

Guinea-Pigs

The disease varies, many of the animals have only shown the parasites after one to three months or more. The trypanosomes have then continued present in large numbers, death occurring fourth, ninth, and sixteenth weeks after the appearance of the parasites in numbers. Rupture of the spleen has caused death in four cases, in every instance the blood was full of trypanosomes and the spleen acutely swollen.

Morphology of the Parasite

Dutton and Todd, Laveran and Mesnil, Bruce, and others have described the parasite. Both Dutton and Todd, Laveran and Mesnil, and Bruce have had opportunity of comparing the trypanosomes found in the blood with those parasites found in the cerebro-spinal fluid and those present in the blood of inoculated animals. All groups of observers have agreed that they can determine no marked differences. All agree that the parasites in the cerebro-spinal fluid are often vacuolated. As Laveran and Mesnil point out and illustrate in their work (Fig. XLII, Nos. 1, 2), and Dutton and Todd (Pl. I, Fig. 1), the trypanosome may contain large vacuoles. Laveran and Mesnil ascribe it to the parasite being poorly fixed, and show that with very serous blood such a condition is common. Such being the case it is only natural that films made from the cerebro-spinal fluid will contain many of these vacuolated forms.

We have compared the trypanosomes in the same way as these observers and can find no difference between the parasite present in the cerebro-spinal fluid and blood of patients,

or in the blood and exudates of infected animals. PLIMMER claims that the parasite of 'Gambia Fever' differs from the sleeping sickness trypanosoma: the former being longer, generally larger, and more easily stained than the stumpy, larger-vacuoled, badly-staining trypanosoma of sleeping sickness. After the examination of many animals infected with the various strains of *T. gambiense* the distinctions which PLIMMER notes appear to be artificial. Both long and short, large and small forms are seen in their blood; on comparing these with the parasite in the blood of a native the same forms are seen. No differences have been noticed in the staining reactions. The parasites in serous blood require longer staining than do those in less anaemic blood.

Comparisons were made by injecting animals both with a sleeping sickness and a 'Gambian Fever' strain. It was impossible to detect any difference whatsoever either in the control films or films made from the animal. Animals were injected with blood containing a majority of short stumpy-formed parasites, these animals have shown as many or more longer forms than stumpy ones in their blood. Careful measurements have not shown any difference between the sleeping sickness or 'Gambian Fever' or any other 'Trypanosome Fever' parasite. These comparison slides were made from the same species of animal, and, with two exceptions, the number of passages of the parasite was the same. Chromatic granulation is common both to the long and short forms. Parasites appear to possess these granules more often if the infected animal is dying or if large numbers of parasites are present in the blood. They have also been prominent in the trypanosomes undergoing degeneration in the blood of an animal treated with arsenic or trypanroth. In some cases they have almost filled up the whole body of the trypanosome, making it difficult to distinguish the micronucleus and interfering with the macronucleus. These granules may form in clusters or may be ranged in comparatively regular rows, they may vary from a pin-point to a size equal to or larger than the macronucleus in size. Trypanosomes containing such granules may be run through several passages and still possess granules. One mouse, Experiment 692, had enormous numbers of trypanosomes containing these granules; all the subinoculated animals became quickly infected, and though this strain was run through eleven passages the parasites still contained the granulations. The granules were present in both single and divisional forms of the micro-organism. These granules were present in the trypanosomes of the subinoculated animals—rabbits, rats, mice, and pup. In a monkey a less number of granules were seen. The twelfth passage into a guinea-pig infected the animal, but the trypanosomes had almost lost the granules. In the blood of this animal granular trypanosomes were noted from time to time; it died from broncho-pneumonia; its subinoculated rats have never shown granular forms of the parasite.

Chronicity

The artificially produced disease in animals may pursue an acute or chronic course. With the ordinary strain all the animals show a more or less chronic and lengthened infection as compared with Nagana and other pathogenic trypanosome infections. It appears more virulent for kittens, pups, and monkeys (*Rhesus*) than for cats, dogs, rabbits, guinea-pigs, rats, and mice ; in some of these animals the parasites, after appearing almost constantly for a time, disappear and are then found only at rare intervals. Such animals usually show an increase of parasites a short time before death. The large animals, goats, sheep, cows, donkeys, and horses, though susceptible to the infection, with the exception of goats, appear to be able to withstand the disease. It is interesting to note that a horse still shows infection twenty-seven months after inoculation.

Virulence

In the majority of animals infected with the various strains of *T. gambiense* the disease pursues a normal course, and the animal dies with a fair number of parasites in its blood. Occasionally the numbers of parasites increase enormously, and such an animal quickly dies. The subinoculations, even with very diluted blood containing no more parasites than control blood, may show that it is extremely virulent, the incubation and duration of the disease being shortened. Such virulency has been noted in baboon 747, rabbit 823, and in rabbits, rats, and mice. Though parasites are numerous in guinea-pigs, in no case has an increased virulency of the parasite been observed. As noted elsewhere if a strain be repeatedly and quickly run through animals of the same species it will acquire a certain virulency for such a species, but this continues only so long as the strain is not run through other species of animals.

Immunity

Certain species of animals appear to possess a more or less stable resistance to infection with trypanosomes. Baboons are peculiarly hard to infect, the immunity is not an absolute one as they have been on occasion infected with *T. gambiense* and *T. dimorphon*. In every case the incubation has been lengthened, the parasites have been scanty but the animals have succumbed to the disease. Certain species of monkeys, as the *Jew* and *Sooty*, are somewhat more difficult to infect than the *Rhesus* and *Calothrix*.

All attempts to infect birds and fish with *T. gambiense* and *T. dimorphon* have failed. Goats[1] and sheep, though susceptible to infection with various pathogenic trypanosomes, do not easily succumb. Some species of animals, ordinarily susceptible

1. As Bruce suggested it is with such animals that the histo-pathology of the disease ought to be investigated. In our report a comparison of the lesions found in animals dying after lengthy period from a chronic type of the Gambian Horse disease has been made with those found in sleeping sickness. A comparative study of a large number of goats, etc., infected would be of great aid in determining if the various trypanosomes pathogenic for animals produce lesions akin to those found in natives dying from sleeping sickness.

to infection, show occasional resistance. One rat, 285 (see p. 25), was difficult to infect with *T. dimorphon*, and then only showed the parasites for a few days. It recovered and was able to withstand inoculations of enormous quantities of virulent blood. In the preliminary note on *T. gambiense* it was shown that sometimes, of two rats inoculated at the same time the one acquired the disease the other did not, the resistant animal even having received the greater amount of the virulent blood.

With these resistant animals and those in which the disease pursues a markedly chronic course experiments have been made to see if an exacerbation of the disease could be produced. Such animals have been daily, weekly, or monthly reinoculated with small quantities of virulent blood. In a few cases a rise of temperature corresponding to the incubation period has been observed. In a couple of cases the parasites have somewhat increased. Various experiments in breeding infected and healthy animals together have been done. The progeny of such unions have not shown any immunity or resistance.

Endeavours to protect animals, or to at least mitigate the disease by injection of attenuated parasites or large quantities of blood from which the parasites have been freed or killed off, have proved of little value. The same applies to injection of animals with sera from animals which have recovered from the disease in which the chronic type prevails, or from animals naturally immune. Here again very meagre results have been obtained. Efforts to produce an immunity by the administration of drugs is of little value. Arsenic, which has an effect on the parasite, is of no use when attempts are made by first treating the animal with the substance to render it later proof against inoculation of virulent blood. Ehrlich and Shiga in their work on 'Trypanroth' were able to protect[1] animals by preliminary treatment with the dye. If large enough amounts are administered a certain amount of protection is afforded.

Toxicity

All efforts to isolate a toxine have proved negative. The various procedures which Plimmer and Bradford had used were employed. They had found that animals could be inoculated with large quantities of blood from dogs infected with Nagana, the blood being filtered through porcelain bougies, without producing any effect. As much as 150 c.c. of filtered serum have been used without producing any toxic effect.

On one or two occasions the injection of blood containing trypanosomes has seemed to us to possess toxic properties. A rabbit infected with *T. gambiense* showed this. The blood as long as there were few parasites present could be inoculated into rats with impunity. Later on, as the parasites became numerous, it was noticed

1. Such a line of investigation with this and other substances ought to be tried on large animals. The history of punitive expeditions, etc., in the past has shown how handicapped man is when a trypanosomic disease breaks out amongst the transport animals. If this dye were administered before passing through a fly-belt, and the animals carefully examined and tested before and afterwards, valuable data would be available.

when the blood was inoculated that rats which received 0·5 to 0·8 c.c. of pure blood died in twenty to twenty-eight hours, while controls injected with a weaker solution, 0·1 to 0·5 c.c., survived. On three consecutive days batches of four rats were inoculated intraperitoneally ; the two animals which received the higher amounts were killed, the other two survived. A repetition of these experiments gave the same results. Cultures made from the rats remained negative, and films from the inoculation mixture, peritoneal cavity and heart blood of the rats, showed no bacteria. This rabbit died a week later. Maceration of its organs and treatment in the usual manner failed to reveal a toxine. The organs of animals dying or killed when numerous trypanosomes were present in the blood and in the juices of the organs, or at any time when the parasites have almost disappeared, have been used. The precipitates obtained from them have neither produced any effect nor afforded any protection against infection. The organs of the two cases of sleeping sickness were tried with like negative results. Peritoneal and pericardial exudates and fluid from oedematous tissues containing parasites have been ground up and injected also with negative results. It is possible, if they could be procured in large quantities, that some substances might be obtained which would cause a reaction, but the quantity of fluid ordinarily obtainable is limited. Centrifugalization of large quantities of blood renders it possible to collect enormous numbers of trypanosomes, but they are mixed with leucocytes. Efforts to get rid of these latter have not been successful. Inoculation of these parasites after they are killed by heat or cold or kept for a sufficient time have failed to produce any effect. From the products of culture tubes no toxine has been found, and animals inoculated with such contents have shown no evidences of intoxication.

Agglutination

The possibility of the agglutination of the parasite acting as an adjunct to the diagnosis of the disease has been realised. The results are not satisfactory. Blood, from an animal showing parasites, when defibrinated and mixed with serum from a native or animal suffering from the disease has been used. The parasites do not agglutinate. Serum from an animal continually injected with living trypanosomes shows the same negative results. In both cases there is a certain agglomeration of the parasites, but this is also usually obtained if normal serum be used. The serum from a native or animal added to defibrinated blood from the same case shows a slight agglutination. The exudate from a bleb causes no definite agglutination. If the trypanosomes be rendered immobile by the addition of weak formol before the serum is added, a partial clumping of the parasites may occur. Brumpt and Wurtz added potassium citrate solution to blood containing parasites to prevent coagulation, and then added serum from a sleeping sickness case. A rapid agglutination of the trypanosomes occurred. As they point out citrated blood containing parasites shows agglutination of the trypanosomes in a short while. In our opinion blood, to which

citrate is added, must of necessity be of no value for agglutination diagnosis on account of spontaneous agglutination of the parasites. With defibrinated blood the objection is that the agglutination of the blood corpuscles seen so often in animals or man suffering from trypanosomiasis renders it impossible to say whether a true agglutination of the parasites is produced.

Serum from the horse, cow, donkey, sheep, or goat, if added in large quantities, produce an agglutination; it is perhaps a trifle more marked if the animals have been infected[1] or cured. Baboon serum agglutinates the parasites to a certain extent, the action seems to increase and remain, as far better agglutinations are observed some hours after the commencement of the experiment.

Conclusions

1. The further comparison of the trypanosomes found in (*a*) the cerebro-spinal fluid of Uganda sleeping sickness cases; (*b*) the cerebro-spinal fluid and blood of the Congo Free State sleeping sickness cases; (*c*) the blood of Congo Free State 'Trypanosome Fever' cases; and (*d*) the blood of Europeans infected in the Congo confirms the previous observations that all these trypanosomes are identical in animal reactions and morphology with *Trypanosoma gambiense* (Dutton).

2. There is no acquired immunity against infection nor transmission of immunity to offspring.

3. That baboons, *Cynocephalus babuin* and *Cynocephalus sphinx*, are susceptible to infection with *T. gambiense*. That usually a chronic type of the infection develops but a fatal termination occurs.

4. That in many of the animals, especially the horse, cow, donkey, sheep, and goat, the infection is of a mild character, but their blood is still infective to susceptible animals one year after the infection. That in the case of the horse the parasites are still occasionally seen and the blood infective twenty-eight months after inoculation.

5. That periodicity of the parasite is a prominent feature both in man and beast.

6. That the passing of a strain from a susceptible into a very resistant animal does not attenuate the organism.

7. That the parasites in an animal may sometimes become more virulent. The numbers increasing enormously, the subinoculated animals become more rapidly infected and death occurs. That such a strain may be particularly virulent for one species of animal. That the more rapid infection is not due to the inoculation of a greater number of parasites than usual.

8. That the parasites, after being passed through many hundreds of animals for nearly three years, still retain its morphological characters, and animals inoculated with it react as described by Dutton and Todd.[2]

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1. The sera from animals infected with other trypanosomata usually cause almost as much.
2. Liverpool School of Tropical Medicine, Memoir XI.

III. *TRYPANOSOMA DIMORPHON.* GAMBIAN HORSE DISEASE

This strain was brought back by Drs. Dutton and Todd from the Senegambia. Our results in general coincide with those of Dutton and Todd and Laveran and Mesnil.

Rats—White and Black

The incubation varies; in the case of the more attenuated strains the incubation period may be as long as twenty days; with the ordinary strain, which has been passed through many rats, the incubation period is from three to twelve days, the average being from four to seven days. Duration of infection, seven to forty-two days; average, eighteen days. Periodicity in some of the rats is fairly well marked. A white rat in the course of thirty-three days showed almost daily an irregular number of trypanosomes in the blood—after amounting to thirty to forty to a field they would diminish to one to thirty to forty fields, and then gradually rise. Each time when the parasites commenced to disappear a marked leucocytosis was present. On account of the smallness of the animal no blood count could be made.

One rat showed a marked resistance to the parasite:—Experiment 285, rat (white), one hundred and eighty-five grammes weight. Inoculated intraperitoneally January 5, 1904, with 0·75 c.c. of blood containing three trypanosomes to a field. Up to February 5, this animal never showed parasites in its blood. It was then reinoculated intraperitoneally with the whole blood from a rat (Experiment 242), there being two parasites to a field. Five days later four parasites were seen to the cover-slip preparation. These continued present for three days; its blood then became negative. On February 22, nine days later, it was once more inoculated intraperitoneally with 3·5 c.c. of pure blood from a dog (Experiment 314), there being twelve trypanosomes to a field in the mixture. It never became infected, nor was its blood infective to small rats when inoculated in quantities of 0·5 c.c. to 0·75 c.c. If some of its serum was added to blood containing the parasites a marked and permanent agglutination occurred. Up to May 10 it was repeatedly inoculated with large quantities of virulent blood, it then succumbed to an epidemic of broncho-pneumonia. Unfortunately, very little serum could be obtained for agglutination and other work. Artificially a certain degree of resistance to this parasite can often be established by injecting a mixture of blood and citrate solution attenuated by preserving in the incubator at 35° C. for four to six hours. Out of seven white rats so treated five of them were able to withstand at the end of eighteen days inoculations of 1·0 to 3·0 c.c. of virulent blood. Their blood, however, caused very slight

agglutination. If these animals were bled very freely, a new inoculation of virulent blood would cause them to become infected, and after that the disease pursued the normal course. The spleens of rat 285 and some of the five others not showing the infection were small and normal looking, the glands were not enlarged. The spleens of rats dying after a duration of more than two weeks are very markedly enlarged, as pointed out by LAVERAN and MESNIL. The glands are also enlarged and many, especially the retroperitoneal group, are haemorrhagic. The pleural surface of the lungs is very often spotted with small petechial haemorrhages. Oedema has only been noticed on two occasions. Paralysis has never been noted in rats dying from the acute form. Two rats suffering from the chronic form developed a partial paresis of the left-hind leg. Haemorrhagic nephritis has been noted in two large rats suffering from the chronic type, the urine contained a few trypanosomes, the kidneys showing numerous small haemorrhages on their surface and in their substance.

MICE—WHITE, BLACK, AND GREY

The incubation varies from two to five days. Death may occur in the acute cases within sixteen to twenty-three days. In the more chronic cases the duration may be from thirty-seven to one hundred and thirty days. In the acute cases the parasites appear and continue to augment almost up to death. In the chronic phase of the disease there is very often a marked periodicity, the parasites being absent from the blood for intervals of eight to fourteen days. Subinoculations at such a time into young rats or mice are negative if small amounts of blood are used.

Two mice inoculated intraperitoneally with blood from Horse VI (p. 30) in amounts of 1·5 to 2·0 c.c., the blood being negative to microscopic examination, never showed parasites until the fourteenth and fifteenth days, the parasites then began to increase and the disease terminated eight days after the appearance of trypanosomes. Two mice were inoculated from the horse at the same time and never became infected, but were later on shown to be immune.

As LAVERAN and MESNIL note, the spleens of mice infected may become enormously enlarged. The organ often being six times as large as normal, causes a most noticeable tumour and almost completely filling the abdominal cavity compresses the other organs. In mice treated with arsenic the splenic tumour has lessened at the same time as the parasites have temporarily disappeared from the blood.

GUINEA-PIGS

Over twenty-five have been used. The incubation has varied between four to fifteen days, the average being four to six days. The duration of the disease is from nine to sixty days. The acute cases showing parasites in large numbers in the blood very often die in thirteen to twenty days. Young guinea-pigs succumb sooner than do adults. There is usually a slight rise of temperature on the appearance of

parasites ; after which the temperature may vary very little from the normal. Loss of weight is usually a feature of the disease. Anaemia is present but is not so marked as in rabbits infected with *T. dimorphon*. Paralysis of the posterior limbs has been seen in two cases about thirty hours before death. The parasites after the first appearance usually continue constantly in the peripheral blood, gradually increasing until there may be forty to sixty to a field. These high numbers may continue for ten days before the animal dies, or diminution in the number coincident with an increase in leucocytes can be noted.

Periodicity in some cases is a prominent feature, the parasites at times almost completely disappearing and remaining so for some days. This feature is observable in the more chronic cases. Rupture of the spleen has occurred in seven cases. Five of these cases were of the very acute form in which, after the incubation period, a continuous and rapid increase of the parasites had been noted. In the remaining two cases the disease was more prolonged. One death happened on the ninth day of the disease ; numerous parasites were present in the blood, and the animal died suddenly. The abdomen contained a large quantity of fluid blood, no lesion of the spleen or other organ could be detected, but the peritoneum was studded with small pin-head sized haemorrhages. One case of haemorrhagic nephritis conducing to the death of the animal occurred. Enlargement of the glands is not marked. If a very attenuated parasite be injected infection may not take place ; the animal is not protected by such an inoculation.

Rabbits

The parasite has gradually become more virulent for these animals. The incubation period after subcutaneous inoculation is longer than after intraperitoneal or intravenous inoculation. An incubation as short as four days and as long as fifteen days has occurred. The average after intraperitoneal injection is nine days, after intravenous four to seven days. Some animals have died in twenty-six to thirty-five days after intravenous inoculation, showing parasites in large numbers in their blood. The more chronic form of the disease can last from seventy-eight to one hundred and fifty-seven days.

These animals show a pronounced periodicity, the parasites being often absent from their blood for four to nine days at a time, to reappear in small numbers and gradually increase to one to ten to a field. Large numbers of trypanosomes to a field, as in the guinea-pigs, are rare. In acute cases the number of parasites is somewhat increased, and they are almost constantly present. In both the acute and chronic types of the disease anaemia is a very marked symptom. Soon after the appearance of the parasites in the peripheral blood the anaemia is noted, this rapidly advances, so that the blood is of a very pale serous character. At the same time a marked loss of weight occurs. In the chronic cases the animals may become mere skin and bones ;

a loss of four hundred to six hundred grammes is not uncommon in these cases. The coat becomes rough. Oedema of the posterior limbs and base of ears may occur to a certain extent. Discharges from the eye, nose, and genital orifices are rarely seen, and the animal never presents the wretched appearance so often seen in rabbits infected with Nagana, Caderas, etc., diseases. Coincident with the appearance of the parasites the temperature rises, and after continuing so for twenty-four hours falls, after that it may become irregular, being often very elevated, and towards the end becoming subnormal. The spleen is enlarged, but not more so than in rabbits dying from Caderas, etc. The glands may be somewhat enlarged, a few are often haemorrhagic, congestion of the organs is often seen.

Monkeys

A *Cercopithecus callitrichus*, a *Macacus rhesus*, a *Jew monkey*, and a baboon, *Cynocephalus sphinx*, have all been successfully inoculated with the disease. The incubation in the *Calothrix* was four days, the duration lasting over one hundred and sixty days. The *Jew* monkey developed the disease on the sixth day, and died seventy-five days after inoculation. In both these animals the parasites were never present in very large numbers until a few days before death. The *Calothrix* showed more parasites and exhibited more symptoms than did the *Jew* monkey. Anaemia, loss of weight, and irregular temperature were noted in both these cases. A baboon, *Cynocephalus sphinx*, ♀, was inoculated intraperitoneally with a large quantity of virulent blood from a dog which had died from the disease on February 22, 1904. On April 18 a rise of temperature was noted, but no parasites could be found. On May 12 eight parasites were found. Unfortunately a daily examination of the blood could not be made, therefore no definite incubation period can be given. From that time the animal rarely showed parasites in the blood until July 4, when they were nearly always present, though in small numbers, up to its death on September 13. The temperature was irregular. Loss of weight was very marked, especially when the parasites were first seen. Anaemia was not a prominent feature of the disease. A *Rhesus* inoculated with some of the baboon's blood developed the disease on the seventh day. Parasites were hardly ever seen, and it finally died from dysentery. A rabbit inoculated intravenously developed the disease after an incubation of eleven days, it is still living, and appears to have recovered from the disease. Two guinea-pigs inoculated at the same time became infected, but never severely, and died from an epidemic of broncho-pneumonia. The serum of this baboon was slightly more agglutinative to the parasite than ordinary baboon serum. Its serum was not protective.

Post-mortem.—The autopsy of the *Calothrix* showed an enlarged firm spleen, glandular enlargement was general, but not very marked. The baboon showed a slightly enlarged spleen and a little enlargement of the glands. The microscopical examination of the brains and organs is referred to by Dr. Breinl.

Cats

Four cats and two kittens have been inoculated. The disease in the adult cat is of a chronic nature. The incubation, after intraperitoneal inoculation, is from twelve to fourteen days. The duration of the disease in three of the animals was nine to ten months, one slightly smaller cat died at the end of forty-six days. The disease pursues a chronic course, the parasites are present only in small numbers, and often are absent for intervals of two to eleven days. The incubation period and the rise of temperature occur together (in one cat, 105·2° F.), after that the temperature falls somewhat but is exceedingly irregular ; after some time the temperature, though remaining irregular, does not rise so high as it did at first. Before death a fall in temperature to subnormal is usual. Loss of weight occurs but the weight is very often regained temporarily ; a month or so before death the loss of flesh is more marked. Anaemia is present but not very marked or progressive. A discharge from the eyes may occur, and in one case oedema around the vulva was noted. The coat is roughened. Two cats which became pregnant after infection aborted. The foeti were examined but no parasites could be found. Small rats inoculated with blood from them never became infected.

A kitten became infected at the end of seven-and-a-half days and died in twenty-three days, the parasites were more constantly present and in greater numbers. Anaemia and loss of weight were marked. Discharge from the eyes and nose was noted. A seeming partial paralysis[1] of the posterior limbs was remarked four days before death. The temperature rose on the appearance of the parasites and remained high but irregular until death.

Post-mortem.—The spleen in the acute form is enlarged. In the chronic form it is only about one-and-a-half times enlarged. The glands are not very big, some few may be haemorrhagic, all contain much juice.

Dogs

Many dogs and puppies have been used. The average incubation after intraperitoneal inoculation is four to eight days, death has taken place in ten to nineteen days. For puppies the incubation is about the same, but the duration appears somewhat prolonged—nine to twenty-six days. The parasites are continuously present in the peripheral blood and increase in numbers, to diminish for a few days. They then begin to augment and usually continue to do so up to or just before death. Loss of weight and anaemia are prominent features. The temperature, after an initial rise, becomes irregular or sometimes continuous ; before death it is usually subnormal.

Dutton and Todd[2] brought back an adult bitch, Experiment No. 11, inoculated October 16, 1902, from Horse I, and infected six days later. This animal, August 31, 1903, was well and strong, its blood non-infective. It is still living, no parasites are

1. While the paralysis is noted no importance is attached to it as young infected animals of all species exhibit such a symptom.

2. Liverpool School of Tropical Medicine, Memoir XI

seen, its blood in amounts of 2·0 to 3·0 c.c. is non-infective. While in England it has been bred and its pups used to see if they were less susceptible than other pups to trypanosome infection. The results show that they acquire the disease quite as easily as other ones, and succumb in the usual time. The serum from the bitch has no protective properties.

Post-mortem.—The spleen is often two to three times larger than normal, the substance is dark and friable, sometimes it may be almost deliquescent. The glands are enlarged, some may be haemorrhagic. Peritoneal exudate is scanty, the pericardium may contain a great deal of fluid. The exudates usually contain a fair number of trypanosomes.

Sheep

A ram was inoculated intraperitoneally. On the sixteenth day the temperature rose slightly and at the same time parasites were seen in the blood. Ten days later ten to forty trypanosomes to a field were noted. These continued in large numbers for the next seven days. The numbers then fell and remained scanty, though continuously present, to death, which took place eighty-four days after inoculation. The anaemia was constant and the animal rapidly became thin. Appetite continued good. No other symptoms were noted. The animal suddenly died during the night. As it was in the height of summer decomposition commenced quickly. The spleen was small, the glands were not markedly enlarged. No oedema or petechial haemorrhages could be found.

Goats

Goat, Experiment 35, inoculated by Dutton and Todd from Horse I, November 28, 1902, remained infected up to March 20, 1903, it was then sent to England. In August, 1903, its blood was negative to microscopical examination, and non-infective even when injected in amounts of 2·0 to 3·0 c.c. It was reinoculated in October and became infected. The temperature rose and remained irregular. Death occurred after inoculation. The parasites were always very scanty until just before death. The autopsy disclosed enlarged spleen and glands, some few of the latter being haemorrhagic. Anaemia was noted a few days before death.

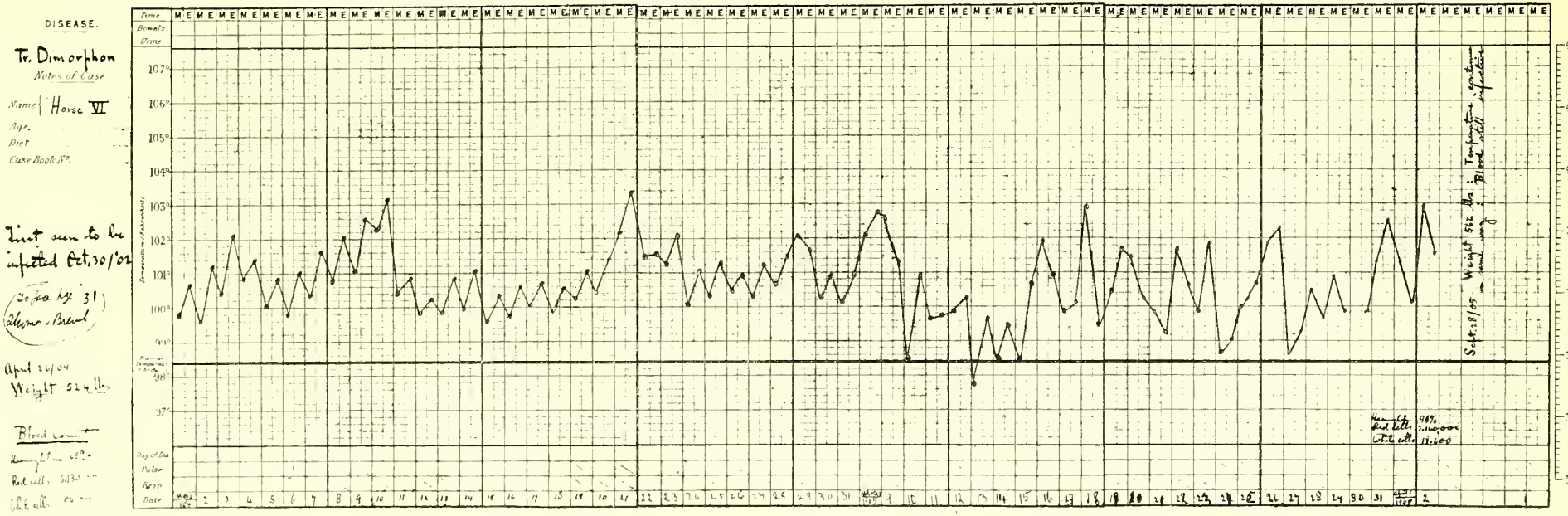
Goat 10, inoculated by Dutton and Todd, October 15, 1902. Appeared to have apparently recovered in October, 1903; in March, 1904, it was killed by accident while being bled. A guinea-pig, inoculated with 100 c.c. of pure heart blood, became infected after a prolonged incubation. The serum from these goats did not cause any permanent agglutination.

Horse

The stallion,[1] naturally infected in the Gambia and sent back by Dutton and Todd, has been under almost continuous observation from October 30, 1902. Since August, 1903, it has been bled at regular intervals, and its blood inoculated into

1. *First Report of the Expedition to Senegambia*, p. 31.

CHART V



to face p. 30

susceptible animals. After two years and five months it is still alive and apparently in good health. *Its blood is still infective to rats and mice*, but the amount necessary in order to secure an infection has had to be increased. Formerly a few drops of blood were required. At present 1·0 to 1·5 c.c. of pure blood is needed to infect a medium-sized rat ; rabbits require 3·5 c.c. of blood. The blood still preserves the characteristic agglutination of the corpuscles. Very occasionally a trypanosome has been found after long continued examination. The temperature has lost the markedly irregular type which characterises the disease. The animal has put on weight and has become accustomed to the change of climate. No symptoms of oedema, etc., have been noted. Blood counts :—April 8, 1905, reds, 7,160,000 ; whites, 17,600 ; haemoglobin, ninety per cent. Its blood no longer causes any more agglutination than does normal horse serum ; the protective properties are nil. The urine is normal.

From the above records it will be seen that observations on the disease are in accordance with the findings of Dutton and Todd and Laveran and Mesnil. The disease in horses can become chronic and the animal may live for some time, probably a cure will result in such a case as the above. From Goat 10 and Horse 6 cases it is evident that animals in which the disease is markedly chronic for some time, will hardly show any evidences of infection. The blood, in amounts of even 1·0 to 1·5 c.c., is non-infective, and when inoculated in larger amounts, 3·0 to 10 c.c., the incubation period is prolonged.

It is not our intention to enter into a detailed description of the parasite as the morphological characteristics have been well pictured and described by Dutton and Todd, Laveran and Mesnil. The parasites have been examined in both fresh and stained specimens from the blood of all the species of animals used in the investigation. In no instance has the long form, described by Dutton and Todd and possessing a long thin body and a long flagellum, been seen ; in this we are in accord with Laveran and Mesnil. The long form is known to have been seen in the blood of animals, but it disappeared early in September, 1903, when the research work in Liverpool was begun. From a study of the films the forms described by Dutton and Todd (Pl. I, Fig. VII, VIII), and Laveran and Mesnil (Fig. XX), are those seen by us. Granules have been seen in many specimens, but not so numerous as in other species of trypanosomes. The agglutination of *T. dimorphon* is a very noticeable feature. As pointed out by Laveran and Mesnil it is especially remarkable in the blood of rats and mice shortly before death, it is also well seen in rabbits and guinea-pigs under the same conditions. In making agglutination experiments with serum, etc., it is therefore necessary to avoid such blood.

IV. *TRYPANOSOMA EQUIPERDUM.* DOURINE—MALADIE DE COIT

This disease has been under observation for some time. Two rabbits which had been inoculated from a dog were received. One rabbit was killed, and the whole of its blood used to inoculate a pup and a rabbit. The pup showed parasites in its blood on the eleventh day, and death occurred on the forty-ninth day, many parasites were found in its blood and in the peritoneal and pericardial exudates. Since that time the strain has been kept running through young puppies and rabbits.

Eight puppies have been used. The incubation period has been from four-and-a-half to eleven days, the average being six-and-three-quarters to seven days. The duration of the disease is from twenty-two to sixty-four days, the average period being from five to six weeks. At first it is extremely hard to find trypanosomes in the blood, but after the third week they quickly increase in numbers, and continue to augment so that eight to ten to twenty-five or more to a field are present. Divisional forms at first are very few in number, but soon abound in the peripheral blood. Profound anaemia occurs coincidently with the increase of parasites. Loss of weight is a marked feature. A characteristic feature of this disease is the oedema of the posterior limbs ; an oedema around the genital organs, extending along the abdomen, is also often very marked, and lasts for a few days or remains till death. In some cases the only oedematous areas are about the site of inoculation. Petechial plaques with loss of hair over these areas have been noted in two cases. Purulent discharge from the eyes and nose very often occurs. There is usually a slight discharge from the genital organs. Nervous symptoms have been rarely seen. In only one pup has a partial but true paralysis of the posterior extremities been noted. This pup developed a halting gait, which steadily became worse, due to an increasing paralysis of the right hind leg. The reflexes were lessened, and towards the end almost completely absent. The autopsy on this pup revealed nothing special, its cord and brain have not been microscopically examined.

One adult dog developed the disease in thirteen days. For a period of three weeks the oedematous condition around the genitals was very marked, the sheath being particularly affected. In addition, small unconnected oedematous areas were present on the inside of the thighs. These oedematous patches contained fluid, in which were very many trypanosomes. The dog lost flesh and became very anaemic, but recovered after two-and-a-half months. Four months later it was killed, and a pup inoculated from its blood developed the disease.

Rabbits

Rabbits inoculated subcutaneously develop the disease in six to eleven days and die in twenty-four to one hundred and eight days, some have lived over six months. The parasites are very scanty during the whole course of the disease. Oedema of the ears and genital organs, very often persisting and increasing, is marked. Loss of hair has been noted on one or two occasions, usually at the base of the ears and around the eyes and nose. The genital organs are swollen, the urethra or vaginal mucous membrane is pinkish and covered with catarrhal exudate. The discharge is often pronounced. The discharges from the eyes and nose are not so marked as in rabbits infected with the parasites of Nagana or Caderas. Examination of these discharges always proved negative.

Rats

Twenty-three rats have been inoculated with large numbers of the parasite ; in only two have parasites been found after the fourth day. One died on the eleventh day, the other on the eighteenth. In neither case could the autopsy be done early, and therefore the negative finding is not to be considered. The spleen and glands were small. A rabbit inoculated with the blood and extract of mashed organs of one of these rats never developed the disease.

Cats

One adult female cat was inoculated at the same time as a pup ; on the ninth day the temperature rose but no parasites could be found. The experiment proved negative.

Guinea-Pigs

Four have been inoculated with negative results.

Goat

A goat inoculated subcutaneously with ten c.c. of heart blood and twenty c.c. of peritoneal exudate never developed the disease. Its temperature remained normal, and its blood negative ; a pup inoculated from it never became infected.

Post-Mortem Appearances

The autopsies on the pups showed a moderately enlarged spleen and enlarged glands, some few of which were haemorrhagic, these being found usually in the retro-peritoneal, inguinal, and axillary groups. The blood was very serous and pale, and usually contained numerous parasites. The bone marrow was pale and often very soft. The pericardial sac contained some slightly clouded fluid. The peritoneal cavity usually contained a large quantity of clear watery or straw-coloured fluid.

These fluids contained trypanosomes often in large numbers. The oedematous patches of tissue contained trypanosomes, often present in greater numbers than in the blood. Petechial patches, the size of a pin's head, were found on the pleural surface of the lungs. The brain and cord did not show any marked macroscopic changes, very often there was considerable oedema around the lumbar region of the cord. The cornea was sometimes opaque.

The autopsies on the dog and rabbits and cat showed no special features. An account of the histo-pathology of the nerve tissues and organs will be found in Part II. Divisional forms of the parasites have been found in all exudate fluid, exudates and juices from the organs. If the autopsy is done immediately after death large numbers of remarkably fine divisional forms will be found in the exudates. These forms rapidly lose their contour if the animal has been dead some hours.

TRYPANOSOMA EQUINUM. MAL DE CADERAS[1]

A large number of animals have been inoculated with this parasite.

Rats—White, Black, and Grey

Rats are easily infected. Subcutaneous inoculation causes the parasites to appear in two-and-three-quarters to three-and-a-quarter days. Intraperitoneal, two to two-and-a-half days. The average duration is six to eight days. In some few cases parasites have been found in the tail-blood in thirty-six hours, the blood is infective at this time.

Mice—White and Grey

Mice are infected somewhat earlier than rats. The duration of the disease is slightly shorter.

In both rats and mice the incubation period and the duration of the disease is lengthened if the inoculated fluid is poor in trypanosomes. Sometimes one notes a temporary lessening in the number of the parasites, this usually lasts for one to three days, sometimes longer. The parasites then increase and usually continue to do so until death. Rats and mice inoculated with blood from animals undergoing treatment with arsenic, especially when the treatment has only been in force for a short period, develop the disease after a prolonged incubation. The parasites may appear for a few days and then disappear to remain absent or once more reappear, such animals are not protected. Loss of weight is noted both in rats and mice when showing marked infection. In some of the rats inoculated with the attenuated parasite the loss has been less marked and of only temporary duration.

Rabbits

The average incubation period has been four to five, sometimes six days. The duration has been sixteen to fifty-nine days. In a few of these animals the parasites have been fairly numerous in the peripheral blood, as many as ten to fifteen to a field being recorded. The majority of the infected rabbits duly show the parasites in very scanty numbers. The parasites are usually found in the blood when the temperature rises. Cases are recorded with a preliminary rise on the fourth to fifth day to 106° to 107° F. The fever is irregular, usually not passing 104° F. A few days before death the rise of temperature may be very marked, death occurring usually with a fall in the temperature. Loss of flesh and a somewhat marked anaemia is noted; this latter symptom is not so marked as in rabbits inoculated with *T. dimorphon* or *T. gambiense*. Oedema at the base of the ears and around the genitals is often marked. Loss of hair around the eyes, nose, and base of ears is usually pronounced.

1. We have been able to study this parasite through the courtesy of Professor Laveran.

The testicles are generally swollen, there usually is a discharge from the penis or vulva. The discharge from the eyes and nose is very severe, causing the animal to present a very miserable state. It has been noted that in treated animals the discharge and oedema quickly disappear, and the hair begins to grow over the bald patches.

Guinea-Pigs

Subcutaneous inoculation causes the parasites to appear in eight to ten days. Intraperitoneal injection takes six to eight-and-a-half days. The duration of the disease may be from twenty-two to one hundred and fifty-three days, the average period being seventy-five to ninety days for adults, and thirty to forty-six days for young guinea-pigs (three hundred grammes). The majority of the animals have shown parasites in their blood almost constantly ; occasionally the organisms nearly completely disappear. This is especially so after a marked increase in numbers. Small guinea-pigs usually harbour large numbers of trypanosomes in their blood. The appearance of the parasites in the peripheral blood is coincident with a slight rise of temperature. The fever then becomes irregular, but not more so than is met with in guinea-pigs infected with Surra, Nagana, etc. Loss of flesh is noted, but is more gradual than in rabbits. Paralysis is rarely observed. Three guinea-pigs showed partial paralysis of the hind extremities, six, three, and one day before death. No guinea-pigs out of forty-three infected and not treated have survived the disease.

Cats

Kittens are more susceptible than adults. The incubation is six-and-a-half to eight days, and death occurs in about thirty to fifty-six days. Adults develop the disease in seven to nine to eleven days, and may die in two-and-a-half to six months. The appearance of the parasites in the general circulation is associated with a rise of temperature. The chart of an infected animal showed an irregular rise and fall with sharp rises to 104° to 105° F. With kittens the temperature usually remains high. The adults rarely show the parasites in their blood, but the agglutination of the corpuscles is often very pronounced. Kittens usually have the parasites almost continuously in the peripheral circulation. The parasites usually are found in the blood at the time of the sharp rises of temperature. Oedema is rarely observed. Discharges from the eyes, nose, and genitals are rare. The autopsy shows a certain enlargement of the spleen. The glands are somewhat swollen. Haemorrhagic glands are rarely seen. In the kitten the gland juice frequently contains many parasites.

TRYPANOSOMA EVANSI. SURRA

This strain was also given by Professor Laveran, its origin is from one of the infected animals in the epidemic which ravaged the island of Mauritius.

A guinea-pig showing a severe infection was received, from it a series of animals have been inoculated.

Rats—White, Black, and Grey

These animals are as susceptible to Surra as to Nagana. The incubation after subcutaneous inoculation has been three to four-and-a-half days, after intraperitoneal injection two-and-three-quarters to three-and-a-half days. The average duration is about five to seven days after the appearance of the parasites in the peripheral blood.

Mice—White and Grey

The incubation after subcutaneous inoculation is about three-and-three-quarters to four days, after intraperitoneal inoculation about three days, duration six to eight days after the infection declares itself. In both rats and mice the parasites usually continue to regularly augment, so that as death approaches large numbers of parasites are found in the peripheral blood. Careful observation has shown that occasionally a decrease in the number of the parasites present in the tail blood is to be noted, it is, however, only transitory, as the numbers quickly increase, so that at death the blood will swarm with the organisms. In rats and mice inoculated with attenuated trypanosomes or with the blood of less susceptible animals, the parasites being fewer in number, the incubation period is lengthened, and the course of the disease prolonged. As in Caderas and the other trypanosomic diseases rats and mice inoculated with blood from animals undergoing treatment, especially where the parasites are still present, though in very scanty numbers and degenerated, may only show a few parasites in the blood. In such cases the parasites are so attenuated that they do not increase rapidly, and the animal acquires a chronic form of the disease. These animals frequently recover, but they are not immune.

Rabbits

The incubation period varies. After intravenous inoculation the parasites appear in three-and-a-half to five-and-a-half days. The presence of the parasites in the peripheral blood is usually accompanied by a slight rise of temperature. Sometimes the rise is marked, being 106° to 107° F., such a high temperature is usually associated with a severe infection. The fever may be marked and of an irregular type, or there may

be hardly any noticeable rise during the course of the disease. The temperature may sharply rise, this is usually in conjunction with an increase in the number of the parasites. The parasites may be noticeably scanty, or they may, as we observed in a few cases, be present in large numbers—ten to forty to a field. Death is usually preceded by a fall in temperature, sometimes the parasites are almost absent before death, at other times they are largely increased in numbers.

The symptoms so often associated with Nagana and Caderas are seen in rabbits infected with the Surra parasites. The oedema of the ears, perineum, the swollen testicles, the tumified vulva, the discharge from the penis, eyes, and nose ; all these are present to a greater or less extent. Anaemia is present but not markedly so. Loss of weight is a constant feature of the disease. Young rabbits are easier to infect than adult ones. The duration is lessened, the parasites are generally more numerous.

Guinea-Pigs

Incubation is six to eight days. Duration, forty days to two to four months. The parasites at first are present in scanty numbers, later in the disease they increase. Sometimes they may almost disappear from the blood and remain so for some time to augment once more. The rise of temperature is usually associated with the appearance of the parasites, the temperature is never very high. Anaemia is not a prominent feature. Loss of weight occurs, being especially marked when large numbers of trypanosomes are present in the blood for some time. Oedema has been noted on a few occasions. No animal has recovered without treatment.

Cats

After intraperitoneal inoculation infection takes place in eight to twelve days. The infection is usually long continued, the parasites are scanty in the blood. The chronic form of the disease in this animal may last six-and-a-half to eight months. There is a certain amount of anaemia associated with a slight loss in flesh ; towards the end these symptoms become more pronounced. A discharge from the eyes and nose has been observed. One cat showed quite a pronounced oedema of the perineum. The disease in kittens manifests a severer form, the parasites are usually numerous, and the duration of the disease may be only four to six weeks. The autopsy shows in these chronic cases very little of interest. The organs appear very little changed. The peritoneal and pericardial exudates, if any, are scanty and usually no trypanosomes can be found. Kittens show an enlargement of the spleen and glands, their blood and peritoneal and other exudates usually contain parasites.

Dogs

Several animals have been inoculated. In all cases after subcutaneous injection the parasites have been found in the peripheral blood in seven to nine days. At first

the parasites are scanty. After a few days marked exacerbations, persisting for a few days, occur ; or the parasite may be scanty until only four to six days before death. The end can occur in sixteen to thirty days, depending on the virulency of the parasite and the method of inoculation. The animal at first presents very few symptoms, but when once the parasites are increasing rapidly a very decided and rapid anaemia, together with emaciation, occurs. The animal continues to lose strength, death is usually preceded by a subnormal temperature. The usual *post-mortem* lesions met with in dogs dying from acute trypanosomic disease are met with.

V. BACTERIOLOGICAL EXAMINATIONS, MORPHOLOGY, GLAND PUNCTURES, AND BLISTERS

Bacteriological Examinations

Cultures of the blood and cerebro-spinal fluid were made from two of the fatal cases of sleeping sickness and several times from ' Native Fever ' cases.

The blood was obtained from one of the arm veins by means of a Roux Syringe —the cerebro-spinal fluid through lumbar puncture. The media used consisted of broth, agar and gelatine of various alkaline or acid reactions. Sugars or peptone, salt, etc., were contained in some media, in greater or less proportions, and were absent from others. Large Ehrlenmeyer flasks containing fifty to two hundred c.c. of nutrient media were inoculated with some of the blood or cerebro-spinal fluid. Anaerobic and aerobic methods of cultivation were employed. The inoculated media were incubated at 18°, 23°, and 35° C.

From Kitambo. Cerebro-spinal fluid, one flask and two tubes showed small growths of *Staphylococcus epidermidis albus.* These were the first cultures inoculated.

From Tomi. All cultures remained sterile. All cultures were kept under observation for two months.

From some of the animals infected with the cerebro-spinal fluid of the two sleeping sickness cases cultures were made at death, the results were negative.

At the *post-mortem* on the two sleeping sickness cases, cultures were made. The blood and cerebro-spinal fluid cultures remained negative. The spleen and liver showed in Kitambo *B. coli communis* and putrefactive bacteria.

From Tomi. No growth.

Variations in the Morphology of the Parasites

From a comparison of stained preparations of *T. gambiense* made during the course of the disease no differences can be made out. The parasites in the sub-inoculated animals seemed somewhat larger. This seemed to be the case with many trypanosomes.

The parasites in the three cases of Trypanosome Fever compared with those found in the blood of the two sleeping sickness cases show no determinable differences. The organisms found in the cerebro-spinal fluid of one case showed a more vacuolic condition, the parasites appeared slightly more rounded at the posterior end. None of the amoeboid forms described by Castellani were seen. The trypanosomes in the blood of the same case did not show the vacuoles so markedly. The observance of many slides of blood, which is often very serous, and of exudates,

causes one not to rely on the presence of vacuoles. They often appear to be present in trypanosomes living under unfavourable conditions. In animals treated with arsenic, vacuolic forms can be seen. If non-vacuolated trypanosomes be injected into the peritoneal cavity of a guinea-pig and some of the fluid withdrawn after a few hours, many trypanosomes containing large vacuoles can often be seen. This is especially so with *T. gambiense*. The vacuoles seem more accentuated in stained films than in the fresh specimens. As LAVERAN points out this is especially observed in slides made from the cerebro-spinal fluid or from very serous blood. Films from such fluids take longer to dry even if the slide be warmed to blood temperature beforehand.

If in a heavily-infected animal the parasites seen in its peripheral blood be compared with those met with in the organs, bone marrow, and large vessels, no differences can be made out. A series of animals were killed and the numbers of parasites present in the organ juices, heart, and large blood vessels compared with the peripheral parasites. In all cases the peripheral blood contained more, the average number of parasites in the different organs were the same. The various groups of glands did not contain more. On comparing the numbers found in the organs of animals which had died from the disease, at from one to twelve hours after death, the results have varied. If the blood be very serous more parasites will be found there than in the organ juices. Sometimes the bone marrow or one organ will contain more than the others. The brain and cord contain fewer than do the organs.

GLAND PUNCTURE—PARASITES IN THE GLANDS

A study of the number of the parasites in the glands as compared with those met with in the blood have not confirmed GREIG and GRAY's observations. From one native superficial glands were removed. Twice were glands taken at a period when the blood was negative to ordinary examination, and once when the blood contained a fair number of parasites. After excision the glands were immediately dried, the surface cauterized, and the gland juice withdrawn. When the blood was negative the glands did not show any parasites; on the occasion when parasites were observable in the peripheral blood the gland juice contained a less number of trypanosomes. Gland juice was also removed by puncture with the same results. Similar findings are to be recorded with glands excised from or punctured in animals infected with the disease. Unfortunately, monkeys, rabbits, guinea-pigs, and rats do not usually have very enlarged glands, but in the other animals no better results can be shown. Gland puncture of two donkeys infected with *T. gambiense* and with very few parasites in their blood have not proved of use in the routine examination of the animals, though the glands were enlarged and easily palpable. DUTTON and TODD have made use of gland puncture with very satisfactory results.

Glands were removed from lightly infected animals—strains, *T. gambiense*, *T. dimorphon*, *T. evansi*[1]—showing parasites in only scanty numbers in the blood. The glands were immediately mashed up with citrate solution and the extract injected into the peripheral cavity of susceptible animals. Eleven experiments were done. In nine the animals were infected only after a prolonged incubation ; in two cases the animals never showed parasites. Controls inoculated with blood and citrate became infected in a shorter time.

Occurrence of Parasites in Blisters

One of the experimental animals having a blister the exudate from the small bleb was noticed to contain a few trypanosomes. By means of cantharides plasters small blisters were made, and the serum was examined from two of the natives and some of the animals. The results were not encouraging. On occasions when the blood was negative the fluid from the blisters was also negative, and on days when the blood was positive only a very small number were seen in the exudate. Sufficient exudate was collected and mice inoculated. After an incubation period of twenty-one days in two out of four mice inoculated intraperitoneally with 0·5 and 1·0 c.c. parasites were seen in small numbers.

Diagnostic Methods

Much attention has been directed to find some method of diagnosis in cases where the blood and gland-juice examinations fail to show parasites; in cases of failure, and when the centrifuged blood is also negative, resource must be had to inoculation. Dutton and Todd record a certain number of their inoculated rats failing to show signs of infection, even when inoculated with blood verified to contain motile parasites. All the rats inoculated from the natives have shown parasites in their blood, yet in some cases the incubation period has been prolonged to thirty-two days. Rats are in many ways unsatisfactory, as the parasites may be present in only very small numbers. Inoculation of larger animals, rabbits, guinea-pigs, and cats are also open to the same objection. Monkeys are most suitable, since they are easily infected ; then come young pups, kittens, rabbits, and adult dogs. Advantage was taken of the periods when the trypanosomes were absent from the peripheral blood of the natives. If after several days the blood still remained negative, small quantities of blood, ten to thirty c.c., were withdrawn from one of the superficial veins and, combined with citrate solution, the mixture was

1. While experimenting with *T. brucei* in Professor Kitt's laboratory, Veterinary School, Munich, and at McGill University, a series of animals were inoculated with extracts from the mashed up organs, glands, bone marrow, etc., of infected animals. Infection quickest with blood mixture, then bone marrow, spleen and liver, kidneys and adrenals, glands, brain, cord. A repetition of the above with *T. gambiense* and other trypanosomes has given almost the same results. Pieces of organs removed aseptically and inserted whole under the skin or in the peritoneal cavity of rats and guinea-pigs have infected the animals after a more or less prolonged incubation period. Pieces of organs left under the skin or in the peritoneal cavity for some hours and then taken out and examined showed trypanosomes more degenerated and in less numbers than in control pieces examined. Celloidin sacs filled with blood containing parasites and placed in the peritoneal cavities of rats and pigs for a time show the usual degenerating, deformed trypanosomes.

inoculated into the animals. These animals became infected, though the incubation period was lengthened. After the appearance of the parasites the disease ran its natural course, and the animal succumbed to the infection in almost the same time as others which had been inoculated with blood in which the organisms were seen.

Monkeys of 1·5 to 2·5 kilo. weight inoculated intraperitoneally with 5·0 to 8·0 c.c. of apparently negative blood always became infected. Kittens can receive two c.c., pups, two to four c.c. of blood intraperitoneally without untoward symptoms developing, and show parasites usually in nine to fourteen days. The kitten and pup are especially useful for studying the disease as they quickly show the parasites in large numbers, and periodicity, particularly in kittens, is not a marked feature. Dogs are also useful, but the number of parasites observed may for a considerable period be small, and the possibility of the unskilled observer passing them by is enhanced.

The Cultivation of Trypanosomes

Novy and McNeal in a series of papers emanating from Professor Novy's laboratory, have shewn that it is possible to cultivate some of the trypanosomes infecting animals, *T. lewisi*, *T. brucei*, *T. evansi*, and a numerous series in birds. We have been able to cultivate *T. lewisi* and *T. brucei* on the media proposed by these investigators. Our attention has, however, been directed more to the artificial cultivation of *T. gambiense*, *T. dimorphon*, and the other pathogenic trypanosomes. If the results must conform to Koch's postulates, then we must admit we have failed. Various strains of *T. gambiense* are capable of being cultivated in a modified blood agar medium and kept alive for sixty-eight days by transference from tube to tube or through flasks ; after the seventeenth day of cultivation we found it no longer capable of infecting a susceptible animal, no matter what amount of culture was used. The trypanosome loses its form to a great extent, its staining reaction is altered and it finally dies. Numerous trials have been made with various changes in the composition of the media but without success. Guided however by the history of the numerous failures of Novy and McNeal before their efforts were rewarded, we believe that our successors will be able to cultivate these parasites with greater success than we have had. That the task is a difficult one is evidenced by the fact that of the many trypanosome investigators only one in addition to those working in Novy's laboratory has been been able to publish a successful result, and this only a repetition of a former investigator's work.

A synopsis of our attempts is included here in the hopes that they will act as a spur to the investigation of this most important subject. As the result of our experience, we feel that the undivided attention of the research worker is necessary to secure success.

After cultivating *T. lewisi*, attention was paid to *T. brucei*, using blood agar in the proportion 2 : 2 and 2 : 1 ; the third attempt proved successful. This culture was run through five generations before it died out from the effects of contamination. Efforts were then directed to *T. equiperdum*, *T. equinum*, and *T. evansi*. The culture of *T. equiperdum* was difficult, as the parasites were not present in large numbers, but at the fifth attempt two tubes out of nineteen inoculated with blood and exudate fluid from a pup, showed motile trypanosomes on the eleventh day. One tube was infected with heart blood, the other with oedema fluid obtained from the subcutaneous tissue of the thigh. The heart blood tube showed evidences of growth and the formation of reduplication forms, but only one in thirty fields, while at the time of inoculation large numbers of these forms were seen. The oedema fluid on the seventeenth day showed many divisional forms undergoing division. These forms were agglomerated together into clumps, not rosettes, as after the manner of *T. lewisi* in culture ; many single well-preserved forms were seen, but the motility of all was much lessened. Many deformed and motionless, granular, degenerated trypanosomes were seen. At this time a pup used for keeping the strain running in the laboratory died. Resource had therefore to be made to the cultures. The total contents were taken and injected into the peritoneal cavity of a puppy. On the eleventh day after the inoculation a slight rise of temperature was noticed, but no parasites could be found. Nineteen days later, *i.e.*, thirty days from date of inoculation, no parasites having been seen it was killed and the whole of its blood inoculated into another pup. This pup after a prolonged incubation became infected. The medium used in this instance was chicken-broth, in the proportion of meat one to water two with 0·5 per cent. peptone and 0·25 per cent. sea-salt added, and rendered faintly alkaline, agar 2·5 per cent., and defibrinated rabbit blood 2 : 1.

T. equinum.—Many failures with this parasite have to be recorded. Cultures were made on various strengths of beef-broth agar, with different proportions of blood, peptone, etc., with no result. Rats, mice, rabbits, and guinea-pigs were used, the animals being killed at all stages of the disease. The trypanosomes quickly died off. One effort yielded a positive result on a chicken-broth agar tube of similar composition to that used for the *T. equiperdum* culture. It was one tube out of twelve inoculated from a rabbit, which had died about three-and-a-half hours before the inoculation of the cultures. The trypanosomes were in the average about one to eighteen fields. The tubes examined showed no growth, after the twelfth day only dead parasites were found. These tubes were left in the incubator at 22° C., and were not examined till the twenty-ninth day, when the majority were found to be overgrown with mould. One was not and it contained a few trypanosomes, many of them being paired, looking apparently healthy with fair motility. A few transplants were made into fresh blood agar tubes, and the rest of the culture was used to inoculate a rat, which became infected after an incubation of eight-and-a-quarter days. The transplants never grew. Further efforts failed to produce cultures.

T. evansi.—Here, as with the other trypanosomes, numerous cultures from the blood of different animals have been made from time to time. Seven tubes at 22° C. showed growth up to the nineteenth and twenty-second days, but these were contaminated with bacteria. One tube showed a growth on the twenty-ninth, another on the thirty-third, still another on the thirty-seventh day. These three latter were all room temperature cultures, the efforts to transplant the twenty-ninth and thirty-seventh day cultures proved failures, nor did the original tubes show any more growth when placed in the 22° C. oven, all the trypanosomes quickly dying off. The thirty-third day tube was used for inoculating a rat and a guinea-pig without success; the few remaining drops were used for sub-cultures, but no growth resulted from them. The trypanosomes in these three tubes, especially that of the thirty-third day, resemble the description which Novy, McNeal, and Hare have given of their efforts to cultivate the Surra trypanosome of the Philippines. The parasites were few in number, were all single and actively motile, the majority showed dark granules at their posterior ends, and resembled the first two drawings of Fig. 1 and the lower of Fig. 2 (Novy, etc.) The flagella seemed longer and thinner than in the normal parasites obtained from an infected animal.

T. gambiense.—The difficulties attending the cultivation of this organism are many. The parasite does not as a rule appear in great numbers in the blood. If an animal is infected with the parasites of Nagana, Surra, etc., once they appear they will augment from day to day until the blood fairly swarms. With *T. gambiense*, on the other hand, rats rarely show very large numbers in their blood, and periodicity being a feature of this disease, the parasites present one day will be found to be absent the next day. This feature is constant in all the animals inoculated. Monkeys, pups, and kittens, however, usually have more parasites in their blood than do the other animals. Advantage as far as possible has been taken of this fact. With the ordinary blood-agar medium no growth takes place, and the trypanosomes die off at once. Veal and chicken-meat infusion has been found to be more suitable than beef infusion. One to 1·5 per cent. peptone with 0·25 to 0·5 per cent. sea-salt is added and agar from 2·5 to 3·5 per cent. Various animals have been used for bleeding—horse, goat, sheep, dog, and rabbit. Almost as satisfactory results have been obtained from the blood of the goat and sheep as from that of the rabbit. The blood has been used in various proportions—2 : 2, 2 : 1, 3 : 1—the most satisfactory proving 2 : 1 or 3 : 2.

The trypanosomes for the first few days appear to tolerate the media, but after the fourth to seventh days numerous degenerated and dead ones are seen. In cultures kept at 22° to 23° the tubes will at the fourteenth to sixteenth day either contain dead trypanosomes or a few partially motile ones. These motile ones may present the normal outline of the parasite, but granules are usually present in the anterior portion of the body. The macronucleus of these forms is often of a granular appearance, staining unevenly, and sometimes fissure-like breaks may

occur. The flagellum is usually shorter, and it and the undulating membrane when stained appear thinner. The motility of some of the forms is accentuated, but usually the motion is markedly lessened. Many parasites are globular and show whitish areas, apparently vacuoles, in the body, and these sometimes encroach on the macronucleus. The motility of these forms is nearly always lessened. The trypanosomes usually occur singly or in two, three or four to a group, sometimes clumps of twenty to thirty will be found, the flagella facing outwards ; these clumps are not composed solely of parasites, but consist of a mixture of blood cells, fibrin, and trypanosomes. Rarely after the twenty-fifth day have normal looking organisms been seen, though at this time there may be found dead trypanosomes with perfectly formed outlines and flagella. If from the sixteenth day onwards attempts be made to transplant the motile trypanosomes into fresh media the majority of the tubes will be failures. A few, however, may survive, and in these, after a few days, the trypanosomes will appear to lengthen out and become still more normal looking. The motility is normal, but unfortunately no duplication forms will be seen. No evidences of actual growth occur, and usually by the twenty-fifth to fortieth day from the first generation the organisms will be found dead. Such cultures naturally do not infect animals even when the whole culture is used to infect one animal. On three occasions tubes which on the twelfth to seventeenth days had looked hopeless, with the degenerating parasites increasing each day, have on the addition of fresh culture fluid from another tube appeared rejuvenated ; some of the parasites appearing to lose their dark granules and to become less stumpy. Twice the contents of such tubes have been poured into other tubes, and the parasites have continued motile up to the forty-third day. The parasites of a first generation tube which behaved in this manner were transplanted into another tube, and were alive, though badly degenerated, up to the fifty-sixth day. They were once more transplanted, but twelve days later were all dead. The original tube had contained motile trypanosomes up to the forty-seventh day.

Various strains of *T. gambiense* have been used. Some of the cultures were inoculated with blood in which the short flagellated and vacuolated form, though associated with the longer form of the parasite, predominated. It has been noticed later on in such cultures that sometimes more vacuolated short forms remained or *vice-versa*. This, though it may not be definite, goes to refute the idea which some investigators have put forward that the short vacuolated form is typical of the parasite found in sleeping sickness cases. With the very virulent strain 'E' some attempts have been made to cultivate the parasites. No living trypanosomes have been found after the nineteenth day. These cultures were non-infective for animals.

T. dimorphon.—A great deal of work has been done with this parasite. Tubes of culture media of the same proportions as in the experiments with *T. gambiense* have been used and the animals from which the inoculating material has been taken

have included rats, mice, guinea-pigs, and rabbits. This parasite has naturally a stumpy form and possesses the peculiarity of a marked tendency to agglutinate in animals near death ; this is especially the case in moribund rats and mice. After inoculation of the culture tubes very little is noted for the first three to four days, and the parasites are usually gathered together in large clumps or several of them are agglutinated side by side or by their anterior ends. The motility is not altered, the form is normal and the parasite stains as usual. Cultivated in the room or at 22° C. changes occur after the fifth day, especially in the tubes incubated at 22° C. The parasites become slower and many show granules. Numerous globular and distorted forms are seen. About the seventh to eleventh days the majority are either motionless or dead. However some very acutely rounded or oval forms, pear-shaped and twice as broad and about half as long as the normal ones, are seen. These latter often recall the 'tadpole' shape, and they may become more rounded. Coincident with these changes larger forms appear, which to a certain extent resemble in size and shape cercomonads. The motility of both these forms is remarkable when compared to the ordinary motion of the parasite ; it is very striking to observe them as they whirl into a field and circle round, often lashing out and going to the side of or leaving the field. Their motion is however, usually circumscribed to the one field. Such forms when stained show a badly staining granular protoplasm, a very small macronucleus and micronucleus. Usually the body of the rounded forms contains small chromatin bodies. The flagellum is thin and only faintly stained, the undulating membrane is hard to make out. In order to stain these forms, longer staining is necessary than with normal trypanosomes. If now, cultures containing these bodies be transplanted, very few of them will survive. Cultures containing these forms are non-infective to animals.

Cultures at 22° C. containing normal-looking trypanosomes have proved infective to animals as late as the twenty-third day, but only when injected in large amounts. By sub-inoculations of the cultures of the very active but degenerated forms, it may be possible to keep the parasites alive to the thirty-sixth day. A second generation tube which had been inoculated with a small piece of the surface of a blood agar flask which had been covered with clotted blood, showed two trypanosomes almost dead, but faintly moving on the seventy-sixth day. This tube was sub-inoculated from a 22° C. flask on the nineteenth day and left sealed up from the thirty-second day. No other parasites than these two could be found.

Summary

1. The parasite *T. equiperdum* in blood agar tubes is capable of infecting a pup up to the seventeenth day, when the tubes are kept at 22° C.

2. The parasite of *T. equinum* is capable of infecting a rat twenty days after the inoculation of the tube. There appears to be an actual growth taking place.

3. *T. evansi* in blood agar cultures at 22° C. is living, but non-infective up to the nineteenth to twenty-second day. Three tubes at room temperature contain motile but non-infective parasites on the twenty-first, thirty-third, thirty-seventh days.

4. *T. gambiense* of any strain is capable of living in a non-infective form in a first culture flask up to the thirty-fifth to fortieth days. Cultures in tubes and flasks, which on the twelfth to seventeenth days contained degenerating parasites, have by the addition of fresh culture fluid regained their vitality. Twice the contents of such tubes by transplanting to a fresh tube have lived to the forty-third day. One lived to the fifty-sixth day, and by a second transplant survived up to the sixty-eighth day.

5. *T. dimorphon* in culture tubes and flasks is infective to animals up to the twenty-third day, when infected in large amounts. Sub-inoculated but non-infective cultures remain alive as long as the fifty-sixth day. In one case, two trypanosomes showing motion were found on the seventy-sixth day.

6. In no case has any evidences of a toxine been noted in the inoculated animals.