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THE CHEMOTHERAPEUTIC ACTION OF PHENANTHRIDINE COMPOUNDS

PART V

THE EFFECT OF FOUR NEW PHENANTHRIDINE DERIVATIVES UPON FLY-TRANSMITTED *T. VIVAX* INFECTIONS IN CATTLE

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In Part I of this work (Brownlee, Goss, Goodwin, Woodbine, and Walls, 1950) a number of new phenanthridinium compounds were described which were at least as active as dimidium against laboratory *Trypanosoma congolense* infections in mice. Four of the best of these compounds were selected for trial in the field, in the hope that one or other of them might show advantages over dimidium in the treatment of cattle trypanosomiasis, either because of greater activity or because of absence of the toxic effects peculiar to dimidium.

The drugs were tested upon the acute stage of tsetse-fly-transmitted *T. vivax* infections in Zebu cattle kept on the plateau of N. Nigeria at Vom. Skilled assistance was limited and our object, therefore, was to obtain the maximum amount of accurate information from a group of animals small enough for us to manage by ourselves. All the observations recorded in this paper, except for the temperatures, were made personally by the authors.

MATERIALS AND METHODS

Phenanthridinium compounds

The four drugs selected were prepared by Dr. L. P. Walls and Dr. T. Dewing. They were as follows:

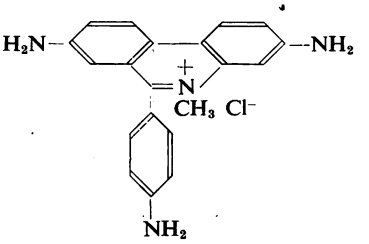
150C47—2: 7-diamino-9-*p*-aminophenyl-10-methylphenanthridinium chloride (1.5 times as active as dimidium in mice).

676C46—2: 7-diamino-9-*p*-nitrophenyl-10-methylphenanthridinium chloride (1.3 times as active as dimidium in mice).

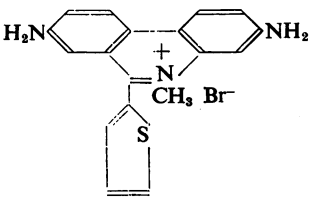
621C47—2: 7-diamino-9-α-thienyl-10-methylphenanthridinium bromide (1.3 times as active as dimidium in mice).

196C48—7-ethoxy-9-*p*-aminophenyl-10-methylphenanthridinium chloride (0.7 times as active as dimidium, and 0.5 times as toxic intravenously in mice).

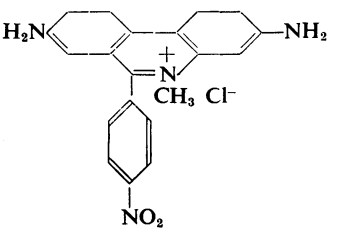
* This work was done while L. G. Goodwin was a guest at the laboratories of the West African Institute for Trypanosomiasis Research from January to June, 1951.



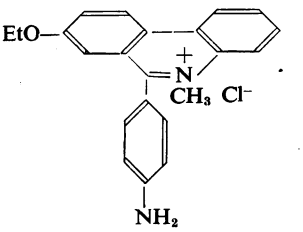
150C47



621C47



676C46



196C48

The drugs were used dissolved in water, and doses were given subcutaneously at the side of the neck. This site was chosen to avoid spoiling the hide from any irritant effects the drug might show. 150C47 was given in 5 per cent solution, 676C46 in warm 2 per cent solution, 621C47 in 1 per cent solution, and 196C48, which was only sparingly soluble, in 2 per cent suspension in water.

For comparison a group of cattle was reserved for treatment with antrycide methyl sulphate at a dose level of 5 mg./kg., injected subcutaneously in 5 per cent solution at the side of the neck.

All solutions were freshly prepared immediately before injection and aseptic precautions were taken.

Fly-transmitted infections—first experiment

Cattle.—The cattle used were the Fulani type of Zebu bulls common upon the N. Nigerian plateau. They were bought at Bokkos by the Veterinary Officer, Mr. Erik Krog, who inoculated them against rinderpest, blackquarter, and haemorrhagic septicaemia before transfer to Vom. All were approximately 18 months old, and weighed between 160 and 260 kg. They were obtained towards the end of the dry season when fodder was scarce; they were lean and in rather poor condition. During the experiment they were fed upon locally-grown hay with no supplements of any kind except for "kanwa" (natron) as a salt-lick. When the grass began to grow at the beginning of the rains, all animals, which were free from trypanosomes in the peripheral blood, were grazed out of doors during daylight; animals which showed trypanosomes were kept indoors in fly-proofed sheds and fresh-cut grass was given when available. By this means we endeavoured to treat all the animals as nearly as possible in the way they would be treated in the field. The animals remained lean until the fresh grass grew and then they put on weight rapidly.

Tsetse flies.—When the experiment was begun, supplies of laboratory-bred tsetse flies were very short and we therefore made use of wild *Glossina morsitans.* The insects were collected through the kindness of Dr. T. A. M. Nash, at Mokwa, Niger Province, on the

Jebba cattle route. Recent experience had shown that about 40 per cent of flies caught in this locality were infected with trypanosomes; both *T. vivax* and *T. congolense* were found in the area, but *T. vivax* predominated. We were fortunate in that the flies we used all produced *T. vivax* infections in cattle, although we were quite prepared to have encountered mixed infections. A total of 46 flies survived the long journey by air and road from Mokwa. They were distributed into 3 in. × 1 in. glass tubes capped with net and labelled individually. They were kept in an insectarium in which the temperature and humidity were controlled. Six weeks later, eight flies remained alive; dissection showed all of these to be free from trypanosomes.

Method of infecting cattle.—It was important to get the 24 cattle infected as uniformly as possible. Therefore, 6 animals were selected at random and each was offered to a batch of tsetse flies. The flies were allowed to feed for 5 min. while the tubes were pressed on to an area of close-clipped skin on the back. On the following 3 days different batches of 6 cattle were bitten, so that by the 4th day all 24 animals had been offered to a group of flies once. A note of the number of flies which gorged themselves at each meal offered was made; each fly fed on the average about every 2nd day, but sometimes probed even when it did not feed. On the 5th day the first batch of 6 animals were bitten again, but the groups of flies were interchanged. The permutations of flies and cattle were continued, so that by the end of 24 days all the flies would have been offered a feed on every beast. However, trypanosomes appeared in the blood of some cattle on the 14th day, and the animals which were infected were taken out of the fly-feeding routine; thus more flies became available for feeding upon the cattle which remained. Three animals already had *T. vivax* present in the blood when they arrived at the laboratory, showing that they had been recently exposed to tsetse fly. These animals were treated in exactly the same way as the rest of the herd and were bitten by the same flies. They were allocated to groups for treatment with drugs on the 14th day, when the first of the remaining cattle became infected. At the end of 30 days of fly-feeding, all the cattle had shown *T. vivax* in the peripheral blood except one (No. 24); this animal had had a rise of temperature 27 days after the beginning of the experiment, but no trypanosomes were detected in the blood. However, a heavy *T. vivax* infection was revealed by lymph-gland puncture taken on the 34th day; the animal had probably become infected at the time of the rise of temperature.

Treatment with drugs.—It was intended that the 24 animals should be divided into six groups of four, so that there would be a group to be treated with each of the new phenanthridinium compounds, a group for antrycide, and a group of controls. When a bull had shown trypanosomes in the blood for 2 days, and had a heavy infection, a dose of a drug was given subcutaneously. The group into which each animal was placed was selected by drawing numbers from a hat. When one animal had been allocated to each of the six groups, a second " round " was started, and so on until all of the animals had been used. In this way, each group contained animals which had developed their infection rapidly, and animals which had taken longer periods to become infected; also the distribution of the bulls between the groups was as impartial as possible. Two animals (Nos. 9 and 10) died before a dose of drug could be given, which meant that two of the groups, which happened to be the controls and the group treated with 196C48, contained only 3 animals each.

When a dose of drug was given, the blood became free from trypanosomes in 1–2 days; after a further few days the treated animal was allowed out of doors to graze on the plateau during the day and to sleep in a kraal at night. If an animal showed a relapse of the infection he was immediately brought into the fly-proofed shed again, to minimize the danger of mechanical transmission to other stock by biting flies. There are no tsetse flies in the neighbourhood of Vom. Animals in which the infection relapsed were given 1 mg./kg. of dimidium bromide on the other side of the neck. When the time came for one of us

(L.G.G.) to return to England, the treated cattle were all free from trypanosomes. They were grazed for a further 3 months, and after a final blood examination sold for slaughter.

Routine examinations.—Weight was estimated by measurement of the girth behind the shoulder with a special tape. The rectal temperature of each animal was taken daily in the cool of the morning. Fresh blood preparations were examined for trypanosomes almost every day, and lymph-gland punctures were taken from all of the animals at the beginning of the experiment and at approximately monthly intervals. The local reaction to the drug was measured and assessed at intervals of a week or two. Punch biopsies of the liver of selected animals were made on several occasions by a method demonstrated to one of us by Dr. Ruth Allcroft, using a trochar and cannula made by A. L. Hawkins & Co., Ltd., of New Cavendish Street, London, W.1.

Fly-transmitted infections—second experiment

A second experiment was begun in June, 1951, at the beginning of the rains. A group of 10 cattle about 12 months old was exposed to infection by *G. tachinoides* collected from Shendam and Jemaa (Plateau province). In spite of frequent exposures for many weeks, none of the animals showed trypanosomes in the peripheral blood. A further supply of the Mokwa *G. morsitans* was then obtained, and with these all but one of the cattle were successfully infected. Some of the animals had mixed infections of *T. congolense* and *T. vivax*. The drugs used in this experiment were 150C47 and antrycide methyl sulphate. Both were given at a dose level of 0.5 mg./kg. so that a direct comparison of activity could be made. Some of the animals were left for a longer period than 2 days from the first appearance of trypanosomes before the drug was given.

Syringe-transmitted T. congolense *infections.*—A small experiment was made with 4 calves about 12 months old, bred at the Veterinary Department Stock Farm at Vom. They were infected with *T. congolense*, transmitted by subcutaneous injection of blood from a heavily infected rat. This strain of *T. congolense* was isolated from a dog in Kaduna in November, 1951, and the 6th rat passage was transferred to the calves. All showed trypanosomes in the blood by the 9th day, and the temperatures were raised. They were treated after 4 days of parasitaemia with 1 mg./kg. of 150C47, 1 mg./kg. of dimidium, 2 mg./kg. of 196C48, and 5 mg./kg. of antrycide methyl sulphate respectively. Blood and lymph-gland examinations and liver biopsies were made as in the first experiment.

RESULTS

The primary infection with trypanosomes was marked by a rise in temperature to 102–105° F. This rise did not occur in two of the three cattle which were already harbouring the infection (Nos. 4 and 5).

The three control animals showed *T. vivax* infections which developed in a manner typical of the disease. One (No. 11) died in the acute phase and one (No. 17) died after 3 months of fluctuating temperature and parasitaemia. The third (No. 15), which was already infected with *T. vivax* on arrival, showed a peak of temperature and trypanosomes in the blood from time to time for 6 months and then passed into a condition of " cryptic trypanosomiasis " (Fiennes, 1950). This animal was eventually killed 12 months after the beginning of the experiment. A succession of small peaks of temperature recurred at intervals of 3 to 4 weeks, and these were associated with the appearance of trypanosomes in larger numbers in the peripheral blood. They suggest some form of cyclical process in the host-parasite relationship.

The results of treatment with drugs are summarized in Table I. The treated animals showed rapid and sometimes profound falls of temperature, which occurred

TABLE I

THE EFFECT OF TREATMENT UPON INFECTED ANIMALS

Group No.	Treatment	Bull No.	Weight (kg.)		Notes
			When dosed	In June, 1951	
I	Controls	11	203	—	Died 8 days after appearance of trypanosomes
		15	203	192	Infected on arrival at Vom. Showed trypanosomes for 6 months from time to time; finally killed 12 months after beginning of experiment
		17	192	—	Died 12 weeks after appearance of trypanosomes
II	150C47 1 mg./kg.	1	181	214	Free from trypanosomes until end of the experiment, 7 months after infection
		7	186	203	
		21	220	316	
		22	230	250	
III	196C48 2 mg./kg.	6	214	—	Died 3 days after dose. Severe local reaction
		8	160	160	Severe local reaction. Relapsed 44 days after dose
		13	210	214	Severe local reaction. Relapsed 15 days after dose
IV	676C46 1 mg./kg.	2	263	293	Relapsed 35 days after dose
		16	220	238	Free from trypanosomes until the end of experiment
		18	190	214	
		19	210	—	Died 17 days after dose
V	621C47 1 mg./kg.	4	214	232	Infected on arrival. Freed from trypanosomes until end of experiment
		24	190	203	Freed from trypanosomes until the end of experiment
		26	220	263	
		29	200	250	
VI	Antrycide methyl sulphate 5 mg./kg.	3	170	—	Died 12 days after dose
		5	250	—	Died 19 days after dose
		23	238	270	Freed from trypanosomes until the end of experiment
		25	200	238	

even in Nos. 4 and 5, which had longer-standing infections and normal temperatures at the time of injection of the drug.

Phenanthridinium compounds.—All the cattle which received 1 mg. per kg. of 150C47 or of 621C47 were apparently cured; they never again showed a trypanosome in the blood or lymph gland juice, and their temperatures remained normal for the rest of the experiment. In addition, the animals put on a considerable amount of weight.

Of the four animals which were given 1 mg. per kg. of compound 676C46, one relapsed and one died. This drug was therefore less effective and possibly more toxic to cattle than 150C47 or 621C47.

All three animals treated with 196C48 relapsed, and two were subsequently cured with 1 mg. per kg. of dimidium bromide. Compound 196C48 was effective in mice and was less toxic by subcutaneous injection than dimidium; we thought

that it might have advantages when given in 2 mg. doses, but this did not prove to be true. Moreover, the substance produced enormous oedematous swellings at the site of injection. The lower subcutaneous toxicity to mice is probably associated with a slower rate of absorption from the site of injection which in turn may be responsible for the more severe local reaction. Although there was considerable variation between individual animals it appeared that 150C47, 676C46, 621C47, and dimidium were about equally irritant at the site of injection. In most of the cattle the drugs produced a hard lump which sometimes gave the animals sufficient discomfort to make them rub their necks on a post or fence. When this occurred the hair was shed and the skin damaged. In the majority of animals all that remained was a patch of bare thickened skin. In three animals, however (Nos. 1, 13, and 22), an area of necrosis was produced which took several weeks to heal. No dressings were applied; the lesions were left uncovered and had all healed at the end of four months. In contrast to these, there were other animals, treated in exactly the same manner, which showed only very slight local reactions. The degree of reaction could not be correlated with the age or colour of the beast, or with any other property we could observe.

Examination of the tissues of the two animals which died showed that No. 6, which succumbed to treatment with 196C48 (2 mg. per kg.), had necrosis of the liver parenchyma around the central veins, and some scattered patches of fatty infiltration. There were also basophilic concretions in the kidney collecting tubules. It is likely that this animal was killed by the drug. No. 19, which died after 1 mg. per kg. of 676C46, had areas of normal liver parenchyma, but the sinusoids and veins were crammed with inflammatory cells. Some areas of the liver were putty-like in consistency, and there were numerous *Schistosoma bovis* worms in the portal venous system. The lungs were congested and the apical lobes consolidated. It seems unlikely that the death of this animal was caused by the drug; the bull certainly had a bacterial infection of some kind.

No treated animal in any group showed evidence of jaundice or of " photosensitization," although it was in the Veterinary Laboratories at Vom that some of the very first occurrences of " photosensitization " after dimidium were observed. The light intensity during the first 3 months of the present experiment was very high; a record obtained from the Meteorological Station at Jos airport, 17 miles from Vom, showed that there was an average of 10 hours' sunshine every day.

Antrycide.—Of the 4 cattle treated with 5 mg. per kg. of antrycide methyl sulphate, two were apparently cured. The other two bulls died 12 and 19 days respectively after the dose. Death followed an illness of a type we had previously observed in cattle which died after injections of antrycide prophylactic mixture, which contains a 5 mg. per kg. dose of the methyl sulphate in addition to antrycide chloride. The chief clinical sign was oedema, which appeared after 6–10 days in the tissues of the anus and the genitalia, later in the lower abdominal wall, and spread finally to the tissues of both hind limbs and the rear half of the body. The urine contained protein. Both animals showed gross oedema of the perirenal tissues and the pelvic wall *post mortem.* There was excessive free fluid in the abdominal, thoracic, and pericardial cavities. Bull No. 5 had also bled profusely into the pelvic tissues and into the abomasum, which contained about a litre of blood clot. Acute haemorrhagic gastritis following toxic doses of antrycide methyl sulphate has also been

reported by Wilson (1949) and by Garner (1950). Burdin and Plowright (1952) have also recorded kidney damage in antrycide poisoning.

Sections of the kidneys of the animals which died showed degeneration of the tubules; many of the collecting tubules were dilated and obstructed with casts (Fig. 1). The glomeruli were not severely affected. The liver and endocrine organs were apparently normal, but the muscle fibres of the heart were filled with fine droplets of fat; both animals harboured *Sarcocystis* in the heart muscle.

The local reaction to injection of antrycide methyl sulphate was less severe than the reactions produced by the phenanthridinium compounds. This was an unexpected finding, because preliminary tests in English cattle showed no significant differences between the irritant properties of dimidium and antrycide (Goodwin and Walls, 1950).

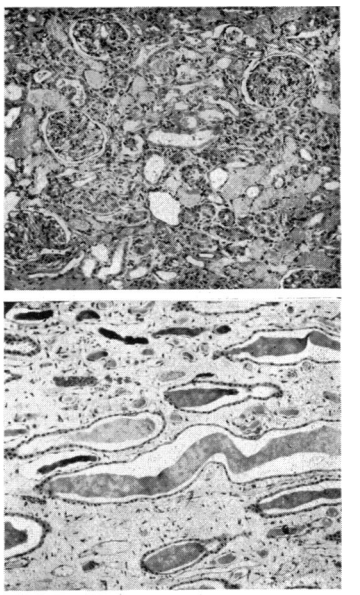


FIG. 1.—Kidney of bull No. 3, which died with severe oedema and proteinuria 12 days after a dose of 5 mg. per kg. of antrycide methyl sulphate. The cortex shows degenerating tubules; the medulla shows the collecting tubules to be dilated and to contain granular casts. Haematoxylin and eosin (× 75).

Unexplained deaths.—Two of the animals died before any drug was given; one of six cattle from the same herd as the test animals, which were neither exposed to tsetse nor treated with drugs, also died. The fact that some of the cattle died before drugs were given makes it necessary to regard with reservation the deaths in the groups which were treated. Fulani cattle which have spent all of their lives out of doors do not take kindly to being shut inside a fly-proofed cowshed. Some pine away and die. However, bulls Nos. 3, 5, and 6 showed definite pathological changes which could be attributed to the action of a poison, so that we must conclude that the treatment made some contribution towards the death of these animals. Fatty droplets in the heart muscle or basophilic concretions in the kidney medulla could not be regarded as reliable evidence of poisoning by drugs, because these lesions were found in untreated control animals.

The results of liver biopsy.—By the method of liver biopsy it was possible to get an idea of the condition of the livers of cattle during the dry season (the time of the lowest level of nutrition) and also to note any effects of the drugs upon the organ when animals have been treated. The specimens obtained showed no gross abnormalities and no changes which could be attributed to the action of any of the drugs given. In Part VI of this work we have studied more fully the toxic effects upon the liver of larger doses of 150C47 (Goodwin and Chandler, 1952).

The second experiment (Table II) showed clearly that at a dose of 0.5 mg. per kg. neither phenanthridinium compound 150C47 nor antrycide methyl sulphate cured *T. vivax* infections. 150C47 was at least as effective as an equal amount of

TABLE II

THE EFFECT OF SMALL DOSES OF 150c47 AND ANTRYCIDE METHYL SULPHATE UPON FLY-TRANSMITTED TRYPANOSOME INFECTIONS

Treatment	Bull No.	No. of days infected before dose	Notes
Controls	54	—	Died 17 days after first appearance of *T. vivax*
	56	—	Alive but infected with *T. vivax* at the end of 113 days
150C47 0.5 mg./kg.	57	1	Original infection of *T. vivax* relapsed 16 days after dose
	58	37	Infected with *T. vivax* and *T. congolense*; died from causes other than trypanosomiasis after 35 days
	59	17	Infected with *T. vivax*; relapsed 19 days after dose
	63	20	Infected with *T. vivax* and *T. congolense*; relapsed with *T. vivax* 22 days after dose
Antrycide methyl sulphate 0.5 mg./kg.	55	1	Infected with *T. vivax*; relapsed 7 days after dose
	60	37	Infected with *T. vivax*; relapsed 22 days after dose
	64	1	Infected with *T. vivax*; relapsed 6 days after dose

antrycide when judged by the period that the animals remained free from trypanosomes in the peripheral blood before they relapsed. The length of time that the infection had been present before treatment did not seem to influence the effect of either drug in the small number of animals studied. A donkey naturally infected with *T. congolense* was, however, cured by a dose of 0.5 mg. per kg. of 150C47; there was no reaction at the site of injection.

The results of the experiment upon syringe-transmitted *T. congolense* infection (Table III) confirmed the conclusions drawn from the experiments upon fly-transmitted *T. vivax*. The animal treated with 196C48 relapsed after 17 days and was subsequently cured with 2 mg./kg. of 150C47. Although the local reaction to the first drug was severe, the second produced only a very small swelling. The calves

TABLE III

THE EFFECT OF PHENANTHRIDINIUM COMPOUNDS AND ANTRYCIDE METHYL SULPHATE UPON SYRINGE-TRANSMITTED *T. congolense* INFECTIONS

Treatment	Calf No.	Weight (kg.)			Notes
		Feb. 7	May 8	June 19	
150C47 1 mg./kg.	I	127	120	127	Free from trypanosomes until the end of experiment, 8 months after infection
Dimidium bromide 1 mg./kg.	III	120	99	123	Free from trypanosomes until the end of experiment, 8 months after infection
196C48 2 mg./kg.	V	99	116	120	Severe local reaction. Relapsed 17 days after dose. Subsequently cured with 2 mg./kg. of 150C47
Antrycide methyl sulphate, 5 mg./kg.	VI	127	124	155	Free from trypanosomes until the end of experiment

treated with 1 mg. per kg. of 150C47, 1 mg. per kg. of dimidium bromide, and 5 mg. per kg. of antrycide methyl sulphate respectively, remained free from trypanosomes in the peripheral blood and lymph gland juice until they were sold 8 months afterwards. The local reaction to antrycide in calf VI was more severe than those observed in the larger animals and took about 6 weeks to resolve. The weights of all the animals decreased during the dry season, but increased again when the grazing improved at the beginning of the rains. Liver biopsy specimens showed no significant pathological changes during the first 5 months of the experiment.

Discussion

The experiments described above showed that two of the new phenanthridinium compounds were effective in the treatment of fly-transmitted *T. vivax* infections in cattle. The other two compounds, although promising in the laboratory, were of little use under field conditions. The animals which relapsed after treatment with 676C46 and 196C48 were subsequently cured by a dose of 1 mg. per kg. of dimidium, which showed that the strain of *T. vivax* used was normally sensitive to this drug. Our main reason for using only a small number of cattle was so that we should be able to make a careful preliminary study of the effects of the drugs upon a uniform fly-transmitted infection. Such a study is of more value than a less carefully controlled experiment upon a larger herd of cattle.

The conclusions to be drawn from the results as they stand is that 150C47 and 621C47 are active drugs with potencies of the same order as that of dimidium. In the dosage used, they caused no serious side-reactions, either immediate or delayed, in cattle which were in poor condition. The effects of larger, toxic doses of 150C47 are described in Part VI of this work (Goodwin and Chandler, 1952). A further study of 621C47 is at present in progress and it remains to be seen whether or not this compound will show any advantages over dimidium. The finding of Burdin and Plowright (1952) that it has considerably lower toxicity to the liver as judged by serum bilirubin, serum alkaline phosphatase, and liver biopsy specimens suggests that it may find a place in the treatment of cattle trypanosomiasis.

Antrycide methyl sulphate at a dose of 5 mg./kg. was effective against the strain of *T. vivax* used, but killed two of the four animals treated because of a toxic effect upon the kidney. When animals are in poor general condition they do not tolerate large amounts of potent medicines. It appears that the margin of safety between the therapeutic and toxic doses of antrycide is not as great as was at first thought, especially when the animals are in poor health. This will be particularly troublesome in areas where strains of trypanosome are encountered which are more than usually resistant to the drug.

Summary

1. Four new phenanthridinium compounds have been tested against tsetse-fly-transmitted *Trypanosoma vivax* infections in Nigerian cattle. Antrycide methyl sulphate was used as a standard of comparison.

2. Two compounds, 150C47 (2: 7-diamino-9-*p*-aminophenyl-10-methylphenanthridinium chloride) and 621C47 (2: 7-diamino-9-α-thienyl-10-methylphenanthri-

dinium bromide) cured infections of *T. vivax* at a dose level of 1 mg. per kg.; doses of 0.5 mg. per kg. were not curative.

3. The other two compounds, 196C48 (7-ethoxy-9-*p*-aminophenyl-10-methyl-phenanthridinium chloride) and 676C46 (2: 7-diamino-9-*p*-nitrophenyl-10-methyl-phenanthridinium chloride), were less active and possibly more toxic. No cases of photosensitization were observed after any of these four compounds.

4. Antrycide methyl sulphate at a dose of 5 mg. per kg. cured two of four cattle. The other two animals died as a result of damage to the kidney tubules by the drug. A dose of 0.5 mg. per kg. of antrycide was not sufficient to cure *T. vivax* infections.

5. It was concluded that 150C47 and 621C47 deserved further study.

We wish to express our thanks to Brigadier J. S. K. Boyd and to Col. H. W. Mulligan for their interest and encouragement, and to Mr. W. G. Beaton, Mr. R. S. Marshall, and Mr. E. Krog, of the Nigerian Veterinary Service, for many acts of kindness in arranging a supply of animals, for some laboratory equipment and facilities, and for the personal accommodation of L.G.G. We also wish to thank Dr. T. A. M. Nash, who made arrangements for the collection and transport of wild *G. morsitans* from Mokwa, and Dr. L. P. Walls and Dr. T. Dewing, who prepared the phenanthridinium compounds.

We are very grateful for the help of Mr. P. E. Nesbitt, who prepared sections of the liver biopsy specimens, and of Mr. H. Richards, who dealt with a large amount of post-mortem material.

Our thanks are also due to our African assistants, especially to Mr. E. Ejemuta for his work in the laboratory, to Mallam Musa II and his herdsmen for the care of the cattle and the daily record of temperatures, and to Hardo Gwon for tending the animals for three months at the end of the experiment.

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