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"ANTRYCIDE"*—A NEW TRYPANOCIDAL DRUG

BY

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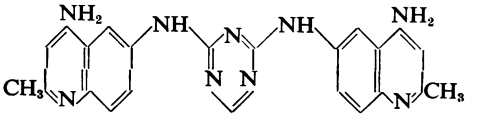
It is generally recognized that the therapy of human trypanosomiasis is at least sufficiently good for the disease to be kept under control, and so the problem of immediate importance in Africa is the control of cattle trypanosomiasis. As much as four and a half million square miles of tropical Africa are considered to be held back from full development mainly by reason of cattle trypanosomiasis, of which the form most widespread, and usually most deadly, is that caused by *Trypanosoma congolense.* Our early work on the chemotherapy of trypanosomiasis was largely influenced, therefore, by the desire to find a drug for use against *T. congolense,* although we did not neglect to look for activity also against other species such as *T. evansi, T. equinum,* and *T. equiperdum* which occur in Africa and elsewhere. So it happened that while early German work on the chemotherapy of *T. congolense* gave us a start in our researches, it was some work of ours with *T. evansi* which led us to "Antrycide." A preliminary account of the discovery was given in *Nature* (Curd and Davey, 1949).

EXPERIMENTAL

If we omit the arsenical and antimonial drugs from consideration the number of trypanocidal substances is few, and limited to the styryl-quinolines, the diamidines, suramin ("antrypol"), the 4:6-diaminoquinaldines, and some phenanthridinium compounds. The activity of these substances has been reviewed by Davey (1949). The class that appeared to be capable of further exploration for our particular purpose comprises the 4:6-diaminoquinaldines. One of them, called "Surfen C," has the constitution (I) (Jensch, 1937)



(I)

It is active against *T. congolense,* and, but for the advent of the phenanthridinium substances, particularly of dimidium bromide (2:7-diamino-9-phenyl-10-methyl phenanthridinium bromide), it might have been used more extensively in the field.

We decided to investigate whether the 4:6-diaminoquinaldine nucleus might be profitably linked with the pyrimidine nucleus, of which an intensive study had been made in these laboratories during our investigation of the chemotherapy of

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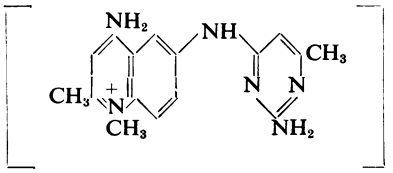
malaria. One of the "quinoline-pyrimidine" substances which was prepared (M4828) was given the constitution (II)

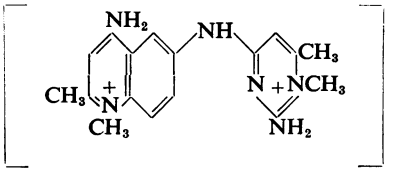


(II)

This substance, or more accurately what we thought was this substance, possessed activity against *T. congolense, T. rhodesiense, T. brucei, T. equinum, T. equiperdum,* and *T. evansi,** but in no instance was the activity sufficiently great to prompt us to suggest a field trial for it.

Pressure of other work then caused us to forsake temporarily this line of research, and we were not thrown back to it for a year or more when some events in the Sudan made us look again at M4828. We were informed by Mr. J. T. R. Evans, of the Sudan Veterinary Service, that results achieved with antrypol in the treatment of *T. evansi* in camels were not so good as formerly and we were asked to suggest another drug. Mr. Evans sent us the strain of *T. evansi* from the Sudan and we tested its response in mice to various substances. The strain was indeed ten to twenty times more refractory to antrypol than two other strains of *T. evansi* we tested, but its response to M4828 was the same. We therefore suggested that the latter drug should be given a field trial, and we prepared more material.

We obtained different results with the newer preparations, and finally, after many checks and counter checks, we were forced to conclude that pure M4828—that is to say, the pure substance having the constitution already given—had only a very poor trypanocidal action, that the much better activity encountered in the original preparation must have been due to an impurity, and that the impurity entered the preparation during quaternization. We thought the impurity must be a substance with pyrimidine nitrogen as well as quinoline nitrogen quaternized, and acting on this belief we prepared M7555 by an unequivocal synthesis. This substance, which is now known as "Antrycide," has the constitution (III) where X is an anion.



(III)

The properties of antrycide

Four salts of antrycide have been prepared, the dibromide (m.p. 316°), the dichloride (m.p. 316°), the di-iodide (m.p. 312°), and the dimethylsulphate (m.p. 265–266°). They are white crystalline solids. The halides are sparingly soluble in water, but the methylsulphate is readily soluble (up to about 33 per cent).

T. vivax and *T. simiae*, important trypanosomes of domestic stock in Africa, were not included in the tests described here because they are non-infective for small laboratory animals.

There is general agreement amongst field workers that injections into cattle are most easily given subcutaneously, and consequently, in our search for a drug to be used against *T. congolense,* we have administered the experimental substances to mice by this route. We found that the much less soluble halogen salts were much less toxic subcutaneously (or intramuscularly) than the freely soluble methylsulphate, whereas intravenously all the salts were about equally toxic (due allowances being made, of course, for the differences in molecular weight). Since the speed of access of a substance to the transport systems of the body considerably influences its toxicity we thought the explanation of the contrasting toxicities must lie in the differing solubilities of the salts, the freely soluble methylsulphate being absorbed rapidly and the poorly soluble halogen salts slowly. Further experiments supported this view. The point is important not only in relation to toxicity, but also to prophylaxis. The greatest service in the control of cattle trypanosomiasis in Africa will be rendered by a drug which not only cures an established infection but confers protection against further infection. We found that antrycide exerted such a protective effect in laboratory animals, and we came to the conclusion that it was due to the establishment of a reservoir or depot of the drug in the subcutaneous spaces (or in the muscles) from which seepage into the transport systems was spread over a comparatively long time. That is to say, the persistence of antrycide in the body, which gives the drug its prophylactic properties, is not due to slow excretion but to poor absorption: inject antrycide chloride intravenously and its prophylactic properties are poor; inject it subcutaneously or intramuscularly and they are considerable.

The experimental details concerning the toxicity, therapeutic action, and prophylactic action of antrycide are given below. Most of the work was done with antrycide chloride and antrycide methylsulphate after preliminary experiments had shown that there was no significant difference between the bromide, chloride, and iodide.

Toxicity

The approximate median lethal dose (LD50), in acute toxicity tests, of antrycide chloride and antrycide methylsulphate for small laboratory animals is given in Table I.

TABLE I

TOXICITY OF ANTRYCIDE IN SMALL LABORATORY ANIMALS

Salt	LD50 of antrycide salts in mg./kg.					
	Mice			Rats	Rabbits	
	s.c.	i.p.	i.v.	s.c.	s.c.	i.v.
Methylsulphate	20–25	15–20	10–15	18–22	*circa* 15	5
Chloride	*		10–15	*	*	

* Not straightforwardly measurable (see text)

Some comment on the toxicity of the chloride administered subcutaneously is necessary. If suspensions of this salt of various strengths, 10 per cent, 20 per cent, and 50 per cent, are prepared and 0.25 ml. of each suspension injected subcutaneously into groups of mice no deaths are caused. If, however, 0.5 ml. is injected some deaths are usually produced, and approximately the same proportion of mice die whatever the total strength of the suspension. Clearly, then, the immediate toxic effects of this salt, when it is given subcutaneously, are related to the amount of drug in solution, and not to the amount in suspension. It follows that relatively enormous quantities of *suspended* chloride can be given to animals subcutaneously or intramuscularly without causing a generalized toxic effect.

A few observations have also been made on the toxicity of antrycide in monkeys and cattle. Two monkeys were given 6 mg./kg. and 7.5 mg./kg. respectively of antrycide methylsulphate intravenously. The one receiving the larger dose collapsed and the other was obviously disturbed; breathing in both was shallow and rapid and both salivated profusely; they recovered within 12 hours. Eight monkeys have been given 10 mg./kg. of the same salt intramuscularly without any apparent adverse effect; one which received 15 mg./kg. was obviously affected and again the breathing became shallow and rapid and the flow of saliva was markedly increased. Cattle which received doses ranging between 12 and 18 mg./kg. subcutaneously exhibited symptoms similar to the monkeys. Details of the experiments in cattle are given elsewhere.

Therapeutic experiments

The results of experiments in which antrycide was used to cure mice infected with various species of trypanosomes are given in Table II. The experiments were done in the following way. Mice were infected by intraperitoneal or intravenous injection of trypanosomes suspended in a mixture of Ringer solution and horse serum. A sufficient number were injected for trypanosomes to be easily found in wet blood smears taken within 24 to 48 hours of infection. Treatment was given not longer than 48 hours after infection as a single dose administered under the skin of the neck. Wet, thick blood smears made from a drop of blood squeezed from the tip of the tail were then examined daily (except Sundays) during a period of 30 days after treatment or until a relapse had been demonstrated. A mouse which remained free of apparent infection for 30 days after treatment was regarded as cured; this standard of cure is somewhat arbitrary but it is sufficient for laboratory purposes. From other work we have done we know that if the strain of trypanosome is well adapted to the mouse—and all the strains used in these experiments were—only a very small proportion of mice (5 to 10 per cent) relapse later than 30 days after treatment.

The doses given in Table II refer to the chloride, although many of the mice were treated with methylsulphate at doses calculated to correspond to chloride. In these and other experiments in mice no significant difference in therapeutic activity between the various salts could be detected if due allowance was made for the differences in molecular weight; this is because the curative doses for

TABLE II

CURATIVE ACTION OF ANTRYCIDE AGAINST VARIOUS SPECIES OF TRYPANOSOMES IN MICE

Numbers indicate the number of mice cured (C) or relapsed (R)

Dose mg./kg. s.c.	*T. rhodesiense* (Tinde)	*T. gambiense*	*T. brucei* (laboratory)	*T. brucei* (Mariakani)	*T. equiperdum*	*T. equinum*	*T. evansi* (Sind)	*T. evansi* (Sudan)	*T. congolense* (Busimbi)
25	8C	10C	6C	7C					
12.5	8C 14R	21C	3C 4R	16C					
5	3C 33R	11C 8R	4C 17R	8C 7R	7C	8C	6C	15C	
2.5	30R	4R	22R	2C 6R	18C	16C	13C	24C 10R	15C
1.25					22C	15C	16C	2C 16R	40C
0.5					21C	7C 6R	5C 7R	23R	7C 26R
0.25					12C 11R	12R			20R

Notes on the strains of trypanosomes

i. *T. gambiense*, *T. brucei* (laboratory), *T. equiperdum*, and *T. equinum* were old laboratory strains, long established in mice, and received by us originally from the Liverpool School of Tropical Medicine.

ii. *T. rhodesiense* (Tinde) was received from Tanganyika in 1946. It has been much used in experiments at the Tinde laboratory. It has been passaged in our laboratory in mice.

iii. *T. brucei* (Mariakani) was isolated from a camel at Mariakani, Kenya, by Dr. E. A. Lewis, who thereafter maintained it in the laboratory by fly passage. We received it in 1948 and have maintained it in mice.

iv. *T. evansi* (Sind) was isolated for us by Dr. Idnani from a horse near Hyderabad in August, 1947. We have maintained it in mice.

v. *T. evansi* (Sudan) was isolated for us by Mr. J. T. R. Evans from a camel in the Sudan in 1946. It is the strain refractory to antrypol. We have maintained it in mice.

vi. *T. congolense* (Busimbi) was sent to us from Uganda in 1946 by Mr. J. B. Randall. The strain had been isolated from naturally infected cattle in November, 1945, and has since been passaged in mice.

mice are so small that even the sparingly soluble halogen salts can be given in complete solution. In larger animals, however, the quantity of drug that has to be administered is such that, for convenience, a salt like the chloride must be given as a suspension. Since the rate of absorption of a suspension of a poorly soluble antrycide salt is very different from that of a solution of a freely soluble salt differences between the curative effect of the chloride and the methylsulphate are to be expected in the larger domestic animals.

TABLE III

COMPARISON OF CURATIVE DOSES OF ANTRYCIDE, ANTRYPOL, AND DIMIDIUM BROMIDE IN MICE

Drug	Approximate minimum curative dose in mg./kg. in mice				
	T. rhodesiense (Tinde)	*T. equiperdum*	*T. equinum*	*T. evansi* (Sudan)	*T. congolense* (Busimbi)
Antrycide* (s.c.) ..	12.5–25	0.5	1	2.5–5	1–2
Antrypol (i.p.) .. .	5–10	2–4	2	25–50†	>100
Dimidium bromide (i.p.)	>25	>25	>25		2–4

* Figures have been " rounded " and can be taken to be applicable to either the methylsulphate or the chloride.

† This strain of *T. evansi* has probably been made resistant to antrypol through mistreatment of camels in the Sudan; other strains are much more susceptible and may be cured by 2–5 mg./kg.

The intensity and range of trypanocidal activity of antrycide are well shown in Table II, and are emphasized again in Table III, where a comparison is made between antrycide, antrypol, and dimidium bromide. Antrycide has the widest range of activity against the various species of trypanosome of any drug we know, and is the most active against *T. congolense*, *T. evansi*, *T. equinum*, and *T. equiperdum*. A point of considerable interest is that we have not succeeded in demonstrating any activity against *T. cruzi*.

Prophylactic experiments

Preliminary experiments were done in mice. Antrycide chloride was injected into the mice subcutaneously, and at varying times after treatment attempts were made to infect them with *T. congolense*. The results of the experiments are summarized in Table IV.

It will be recalled that the toxicity experiments we did in laboratory animals had shown fairly conclusively that antrycide chloride is poorly absorbed from the subcutaneous spaces, and this led us to think that the prophylactic powers of the drug, which are clearly shown by the results in Table IV, might be dependent on the establishment of a store of unabsorbed drug rather than on its persistence

TABLE IV

PROPHYLACTIC ACTION OF ANTRYCIDE IN *T. congolense* INFECTIONS IN MICE

The period in weeks is the period elapsing between treatment and attempted infection; the negative sign indicates that the given number of mice resisted infection, the positive sign that they became infected.

Dose mg /kg.	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks
25	10−	8−	6−	7−, 1+	1−, 2+
12.5	8−	8−	1−, 6+	8+	
5	3−, 7+	8+	8+		

in the body in the usually accepted sense of the term. Two experiments that we did proved the essential correctness of this view.

Experiment (*a*). A suspension of antrycide chloride can be injected beneath the skin of the back of rabbits in such a way that the bulk of the drug is localized in a very small area, where it eventually becomes encapsulated. Rabbits which have received 5 mg./kg. in this way resist infection with *T. congolense* for at least two months afterwards. If, however, the drug is removed surgically the rabbits can be infected within a month.

Experiment (*b*). It can be seen from Table IV that mice treated with 25 mg./kg. antrycide chloride resist infection with *T. congolense* for at least six weeks afterwards. If the same amount of drug is given intravenously by a slow injection spread over about half an hour many of the mice can be infected seven days later.

Discussion

The more important results of the experiments outlined above were communicated to the Colonial Office Tsetse Fly and Trypanosomiasis Research Committee and to the Veterinary Department of the Sudan Government, and with their co-operation arrangements were made for preliminary field experiments to be done in the Sudan and East Africa. Two salts, the methylsulphate and the chloride, were chosen for these experiments. We expected the methylsulphate, because of its much better absorption, to be superior curatively and the chloride, because of its poor absorption, to be superior prophylactically. We felt that our laboratory experiments gave us a reasonably accurate guide to the region in which the size of the curative dose of methylsulphate in domestic animals might lie, but we could not forecast the prophylactic results, for two reasons. In the first place some precipitation of antrycide chloride might follow the injection beneath the skin of antrycide methylsulphate through interaction of the salt with body fluids containing chloride ions, and the amount of this precipitation, which could not be guessed, would determine the degree of prophylactic power possessed by the methylsulphate. Secondly, the prophylactic power of the chloride seemed to depend in some measure on the size of the host. Only results achieved in mice have been quoted above, but in other experiments in rats and rabbits longer periods of protection were given by comparable doses. We were hesitant, therefore, to forecast what might happen in cattle and other large animals.

Our African experiments were designed to embrace cattle trypanosomiasis and (in part) *T. evansi* in camels, but clearly our laboratory results show that antrycide is fully worthy of trials against infections due to *T. equiperdum* and *T. equinum*.

Addendum

The experiments concerned with antrycide itself which are described above were done during 1947 and the early part of 1948, but for various reasons this account of them could not be prepared until September, 1949. In the interval Dr. A. Spinks, a colleague in these laboratories, studied chemically the absorption and excretion of antrycide in various animals and has fully confirmed the inferences we drew from purely biological experiments.

SUMMARY

1. An account is given of the work which led to the preparation of " Antrycide."

2. Two salts have featured in the experiments—antrycide chloride, which is only slightly soluble in water, and antrycide methylsulphate, which is freely soluble in water. Absorption of the two salts, after their subcutaneous injection, appears to be directly related to their solubility ; a suspension of the chloride is absorbed slowly and a solution of the methylsulphate rapidly.

3. A table is given showing the curative properties of antrycide in mice infected with various species of trypanosomes. The substance is most active against *T. congolense, T. evansi, T. equinum,* and *T. equiperdum,* but also exhibits marked activity against *T. brucei, T. rhodesiense,* and *T. gambiense.* No activity has been detected against *T. cruzi.*

4. It is shown that antrycide may be used to protect mice for several weeks against *T. congolense.*

5. Evidence is quoted to show that the prophylactic properties of antrycide are due, for the most part, to the establishment of a reservoir of drug beneath the skin from which absorption takes place slowly, and not to persistence of the drug in the body in the usually accepted sense of the term.

The chemical work leading to the synthesis of antrycide was carried out with the co-operation of Dr. A. D. Ainley, Dr. P. A. Barrett, Dr. G. E. Beattie, Mr. W. Hepworth, Dr. A. G. Murray, and Dr. C. H. Vasey. A full acount of this work will be published elsewhere.

We are indebted for help with the biological work to Mrs. Margaret Smith. Mrs. Joan Nellist, and Mrs. June Massey.

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