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THE ACTION OF ANTRYCIDE UPON TRYPANOSOMES *IN VITRO*

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This paper reports experiments on the action of antrycide upon normal and resistant trypanosomes *in vitro*.

METHODS

The stock strain of *Trypanosoma equiperdum*, which has been maintained at this Institute for about thirty years, was used. An antrycide-resistant strain was prepared from it by treating infected mice with one or several sub-effective doses of antrycide and then passing the trypanosomes into fresh mice, and so on. After about six months, a high degree of resistance *in vivo* was obtained; a dose of 0.4 mg. antrycide/20 g. produced practically no effect upon the development of the infection, whereas with the normal parent strain 0.05 mg./20 g. was sufficient to clear the blood of trypanosomes.

The *in vitro* experiments were carried out by the technique of Yorke and Murgatroyd (1930) with some modifications. The medium consisted of equal parts of rabbit or horse serum and of Tyrode solution adjusted to approximately *p*H 7.2. This was distributed in 0.5 ml. amounts into small flat-bottomed tubes, closed with rubber bungs. Appropriate concentrations of antrycide methylsulphate were added to the Tyrode solution, three tubes being set up for each concentration. Approximately 0.05 ml. of washed trypanosome suspension was added to each tube, and the tubes were then incubated at 35° C. In order to test the infectivity of the trypanosomes for mice, the contents of a tube were injected intraperitoneally into one or two mice. The quantities of antrycide carried over with the inoculum were too small to have any significant effect.

RESULTS

Action Upon Normal Trypanosomes

During the course of many experiments trypanosomes (*T. equiperdum*) were consistently killed in 20 hr. by concentrations of antrycide ranging from 10^{-6} to 10^{-7}. Trypanosomes were not killed by these concentrations in 12 hr. When the period of observation was extended to 48 hr., the apparent trypanocidal power of the drug was not greatly increased. When trypanosomes were exposed to low concentrations of antrycide for a long enough period and then injected intraperitoneally into mice (approximately 100,000/mouse) the mice did not become infected although the trypanosomes were actively motile at the time of injection and although trypanosomes from control tubes without drug were normally infective. (This loss of infectivity is a common phenomenon when trypanosomes are exposed *in vitro* or *in vivo* to antrycide, suramin, or phenanthridinium compounds.) In the present experiments this loss of infectivity appeared to be a gradual process depending upon the length of exposure and the concentration of antrycide. Thus, in one experiment when the trypanosomes were exposed for 5 hr. at 35° C., the minimum concentration of antrycide which destroyed infectivity was 4×10^{-6} to 10^{-6}; but when they were exposed for 24 hr. the minimum effective concentration was 10^{-9}. In other experiments infectivity was destroyed when the trypanosomes were exposed for 1 hr. before injection although not if they were exposed for only half an hour. The gradual nature of the combination between drug and trypanosome was also demonstrated by experiments in which the trypanosomes were exposed to antrycide for a suitable period and then washed and set up again in nutrient medium at 35–37° C.; when the trypanosomes were removed from the antrycide (10^{-6}) in 6 or in 12 hr., they survived *in vitro* for a further 20 hr.

Action Upon Antrycide-resistant Trypanosomes

The action of antrycide upon normal and resistant strains *in vitro* is shown in Table I. The sensitivity of the two strains to the direct trypanocidal action of the drug was almost the same. Thus, the minimum trypanocidal concentration at 24 hr. was 10^{-6} to 2.5×10^{-7} for the normal strain and 4×10^{-6} to 10^{-6} for the antrycide-resistant strain, giving a ratio between the two strains of about 4. On the other hand, there was a marked contrast in the retention of infectivity by the two strains. After 5 hr. exposure the infectivity of

TABLE I

THE EFFECT OF ANTRYCIDE UPON TRYPANOSOMES *IN VITRO* AT 35° C.

Strain	Concn. of Antrycide	Trypanosomes					
		Start	5 Hr.		24 Hr.		48 Hr.
		No./mm.³	No./mm.³	Infectivity	No./mm.³	Infectivity	No./mm.³
Normal	1:250,000	56	84	0/2	0		
	1:1 million		59	1/2 (11)	0		
	1:4 ,,		60	2/2 (8, 8)	3	0/1	0
	1:16 ,,		60	2/2 (3, 3)	44	0/1	22
	1:64 ,,		60	2/2 (2, 3)	62	0/1	33
	1:256 ,,	50	60	2/2 (2, 2)	56	0/2	25
	1:1024 ,,	72	45	2/2 (2, 2)	31	0/2	22
	Control	73	86	2/2 (2, 2)	53	1/1 (7)	16
Antrycide-resistant	1:250,000	27	19	2/2 (3, 4)	0		
	1:1 million	27	19	2/2 (2, 3)	1	0/1	
	1:4 ,,	27	31	2/2 (2, 2)	16	1/1 (9)	Contaminated
	1:16 ,,	27	20	0/2 (2, 2)	17	0/2	5
	1:64 ,,	27	20	2/2 (2, 2)	25	1/1 (6)	0
	1:256 ,,	27	20	2/2 (2, 2)	5	0/1	Contaminated
	Control	27	32	2/2 (2, 2)	23	—	14

The figures for " Infectivity " indicate the proportion of inoculated mice which became infected, and also (in parentheses) the number of days before trypanosomes appeared in their blood.

the normal strain was mostly destroyed at 4×10^{-6} and 10^{-6} and much diminished at 2.5×10^{-7} whereas the infectivity of the resistant strain was not appreciably affected by 4×10^{-6}. After 24 hr. the infectivity of the normal strain was destroyed by 10^{-9}, but some of the resistant trypanosomes were still infective after exposure to 2.5×10^{-7}.

DISCUSSION

According to the experiments described above, antrycide is effective in killing our strain of *T. equiperdum in vitro* at 35° C. at a concentration of 10^{-6} in 20 hr., and under our conditions it destroys their infectivity for mice in much lower concentrations. These findings differ considerably from those reported by Ormerod (1951) working in this laboratory with the same strain of trypanosomes. He found that the minimum trypanocidal concentration after 20 hr. was 5×10^{-5} to 1.25×10^{-5}.

The difference in the results is difficult to explain. In our experiments antrycide-resistant trypanosomes were almost as sensitive as the normal trypanosomes to the *in vitro* trypanocidal action of antrycide—this agrees with the similar finding by Ormerod, although at a different concentration. On the other hand, resistant trypanosomes were much less sensitive than normal ones to the effect of antrycide upon infectivity. In view of this behaviour of antrycide-resistant trypanosomes it is considered that the direct trypanocidal action of antrycide *in vitro* is probably not the explanation of its therapeutic effect *in vivo*, even though it takes place at concentrations comparable to those which are achieved *in vivo* (Spinks, 1950). On the other hand, the action of antrycide in causing loss of infectivity is probably important for its therapeutic action. Similar loss of infectivity is caused by suramin and phenanthridinium compounds, which resemble each other in their therapeutic effects. Perhaps these compounds suppress the multiplication of the trypanosomes.

SUMMARY

1. When trypanosomes (*T. equiperdum*) were exposed to antrycide methylsulphate *in vitro* at 35° C. for 20 hr., the minimum trypanocidal concentration of the drug was 10^{-6} to 2.5×10^{-7} for a normal strain, and only four times greater than this for an antrycide-resistant strain, which resisted the maximum tolerated dose *in vivo*.

2. The power of normal trypanosomes to infect mice was destroyed by *in vitro* exposure to antrycide 10^{-6} for 5 hr. at 35° C., but that of antrycide-resistant trypanosomes was hardly affected by 4×10^{-6}.

3. Judging by these results, the power of antrycide *in vitro* to destroy infectivity is probably more significant for its therapeutic action *in vivo* than is its direct trypanocidal action *in vitro*.

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