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THE EFFECT OF NITROFURAZONE ON *TRYPANOSOMA RHODESIENSE* INFECTIONS IN MICE

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Mice infected with a freshly isolated strain of *Trypanosoma rhodesiense* were not cured by ten daily intraperitoneal doses of 50, 100, 200, or 400 mg./kg. of nitrofurazone (5-nitrofurfuraldehyde semicarbazone). At the highest dosage used, the drug was lethal to some of the mice.

Nitrofurazone (5-nitrofurfuraldehyde semicarbazone; Furacin) was found to cure mice (Dodd, 1946; Giarman, 1951) and rats (Dodd, 1946) infected with *Trypanosoma equiperdum*. Dodd (1946) found that 150 mg./kg. cured 73.3% of the mice, while Giarman (1951) estimated the curative dose for 50% of the mice (CD50) to be 247.6 mg./kg. when given in a single subcutaneous injection and 263.7 mg./kg. orally. Packchanian (1955) showed that total quantities of 450 and 600 mg./kg. orally in nine or twelve daily doses, respectively, cured all mice infected with *T. gambiense*. Evens, Niemegeers, and Packchanian (1957a) found that 16 out of 32 guinea-pigs infected with various strains of *T. gambiense* were cured by single intramuscular doses of from 50 to 150 mg./kg. of nitrofurazone. However, only 3 out of 30 guinea-pigs infected with strains of *T. rhodesiense* were cured by similar doses. Evens, Niemegeers, and Packchanian (1957b) demonstrated that nitrofurazone was of some value in treating human cases of *T. gambiense* infection. The compound did not appear to cure *T. cruzi* infections (Packchanian, 1957).

The lethal dose for 50% of the animals treated (LD50) has been variously estimated as 545 mg./kg. (Dodd, 1946) and 735.8 mg./kg. (Giarman, 1951) orally to mice, 753.0 mg./kg. (Giarman, 1951) subcutaneously to mice, and about 450 mg./kg. (Evens *et al.*, 1957a) orally to rats.

In view of the above work it seemed worth while to determine the effect of nitrofurazone on a recently isolated strain of *T. rhodesiense*.

MATERIALS AND METHODS

Strain.—Strain No. SS 284 was isolated in albino rats inoculated with the blood of a Nyala fisherman believed to have become infected on Sigulu Island in the north-east corner of Lake Victoria, East Africa. The patient had been ill for four months before the isolation of the strain. The strain caused an acute infection in albino rats and was diagnosed on these and clinical grounds as *T. rhodesiense* Stephens and Fantham.

Animals.—Male albino mice (average weight 24 g.) of the closed colony maintained by the East African Trypanosomiasis Research Organization at Tororo, Uganda, were used.

Drug.—Nitrofurazone (5-nitrofurfuraldehyde semicarbazone; Furacin), kindly supplied by Messrs. Smith, Kline, and French, was prepared for inoculation by grinding 0.75 g. of drug with 0.25 g. of gum acacia in a mortar with 25 ml. distilled water to give a suspension of 3% (w/v) of the drug in a 1% (w/v) gum acacia solution. (On the first day, 0.5% gum acacia solution was used.)

Conduct of Test.—Blood from one of the original isolation rats was diluted with heparinized 5% glucose solution, the dilution being adjusted following a haemocytometer count so that about 1,000,000 motile trypanosomes were present/0.1 ml. Eighty-five mice were each inoculated intraperitoneally with 0.1 ml. of this suspension. Ten uninfected mice were also used. Fresh preparations of the tail blood of infected mice were examined daily (except Sundays); if trypanosomes were not seen in 20 fields of these preparations (magnification about $\times 400$), the mice were classed as negative. Treatment was commenced in all groups on the sixth day after the mice which had been infected received their inocula. At this time all these mice had at least five, and usually more, trypanosomes in each microscopic field of their blood. For treatment, the surviving infected mice were divided at random into four groups of 15 to 17 individuals and one group of five. The uninfected mice were divided into two groups of five. The treatment of these groups is shown in Table I. Nitrofurazone suspension was given intraperitoneally in 10 equal daily doses on the basis of the average body weight of each group of mice: those in group 1 received 0.04 ml. of suspension each; group 2, 0.07 ml.; group 3, 0.14 ml.; and groups 4 and 7, 0.29 ml. The gum acacia solution

TABLE I

DETAILS OF THE ADMINISTRATION AND EFFECT OF NITROFURAZONE SUSPENSION, AND ITS DILUENT ALONE, ON MICE INFECTED WITH *T. RHODESIENSE* AND ON UNINFECTED MICE

The doses indicated in treatment column were given daily for 10 days. * Indicates that only those mice which died with parasitaemia after their treatment had ended (in groups 1 to 4) were included in the calculation. † Indicates inclusion of one mouse which died 4 days after its treatment ended without having relapsed. ‡ Indicates that there were no further deaths in these two groups within the next 42 days.

No. of Group	Treatment	No. of Mice				Survival Time after Infection (Days)*	
		At Start of Treatment	Dying during Treatment	Dying with Parasitaemia after Treatment	Cured (Surviving without Parasitaemia 42 Days after Treatment)	Mean	Range
Mice infected with *T. rhodesiense*							
1	Nitrofurazone, 50 mg./kg.	17	3	14	0	40	34–52
2	,, 100 ,,	16	4	12	0	39	30–49
3	,, 200 ,,	15	7	8	0	52	38–69
4	,, 400 ,,	17	15†	2	0	48	42 and 54
5	1% gum acacia, 0·3 ml. ..	5	2	3	0	19	13–27
Uninfected mice							
6	1% gum acacia, 0·3 ml. ..	5	0‡				
7	Nitrofurazone, 400 mg./kg.	5	2‡				

was given to mice of groups 5 and 6 intraperitoneally in 10 equal daily doses. Group 5 was intended as a control of the virulence of the strain of trypanosomes, group 6 as a control of the toxicity of the gum acacia solution, and group 7 as a control of the toxicity of nitrofurazone to uninfected mice.

RESULTS

The results are shown in Table I. The drug cleared trypanosomes from the blood of all treated mice after two or three doses, but the mice which survived the course of treatment redeveloped parasitaemia between 6 and 23 days later (except one mouse in group 4, which died without having relapsed 4 days after its treatment ended) and subsequently died with high parasitaemia. However, the mean survival times of all groups of treated mice were increased as compared with the untreated infected mice of group 5. Two uninfected mice of group 7 died during their course of treatment presumably due to poisoning by the drug.

DISCUSSION

It is clear from Table I that nitrofurazone, given in ten equal daily doses of 50 to 400 mg./kg., failed to cure any mice infected with a recently isolated strain of *T. rhodesiense*. The infections were, however, temporarily suppressed, and the treated mice survived longer than the untreated controls.

The maximum dosage given in this experiment was apparently lethal to two of five uninfected mice in group 7, after 4 and 6 doses respectively: the survival of all the mice in group 6 suggests that this toxicity could not have been due to the gum acacia solution in which the drug was suspended. The progressively increasing numbers of mice which died during treatment in groups 1 to 4 suggest that the drug may be more toxic to infected than to uninfected mice. Some of the deaths may have been due to trypanosomiasis, but if this were the only explanation then a higher death rate in the groups given the lower dosages would be expected.

These results differ, as far as curative power is concerned, from those of Evens *et al.* (1957a), who cured 3 out of 30 guinea-pigs infected with *T. rhodesiense* with single intramuscular injections of from 75 to 150 mg./kg.. of nitrofurazone. The difference may be due to the different host, to differences in the strains of trypanosomes used, or to the route of administration of the drug. The infections treated by Evens *et al.* (1957a) were more chronic than those used in the present work, the average life of the infected guinea-pigs being about 50 days. As recently pointed out by Ashcroft (1958), the albino mouse may not be a very suitable animal for determining the curative powers of drugs in human trypanosomiasis, but it is often the only animal which can be used in large numbers: the results of such experiments can be, at best, only a guide to the behaviour of the drug in human infections.

If used therapeutically, nitrofurazone would be given orally and not parenterally as it was in this test. However, Giarman (1951) showed that estimates of the effective doses in 50% of the animals were similar whether the drug was given orally or subcutaneously, as regards lethal and curative properties. Packchanian (1955) found similar therapeutic results whether the drug was given orally or intraperitoneally to mice infected with *T. rhodesiense*. Thus the route of administration does not seem to affect the absorption of the drug greatly.

Taking into account the results of Evens *et al.* (1957a) and those reported in the present paper, the use of nitrofurazone in the treatment of human infections with *T. rhodesiense* does not seem very promising. However, it appears to be a relatively non-toxic compound and a small trial of its effect on human cases might be justified.

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