VARIATION IN CHEMOTHERAPEUTIC SUSCEPTIBILITY ASSOCIATED WITH CHANGE IN VIRULENCE OF A STRAIN OF *TRYPANOSOMA BRUCEI*[1]

By C. H. BROWNING and R. GULBRANSEN

From the Pathology Department of the University and Western Infirmary, Glasgow

It is well known that the responses to a trypanocidal agent may differ greatly with various species of trypanosomes and even with different strains of the same species in experimental infections in a particular host. Also it may be stated generally that in animals such as mice infections caused by trypanosomes which are not acutely pathogenic for the host tend to be less readily sterilised by drugs than are those which are acutely fatal. Thus *T. brucei* on repeated passage through mice rapidly becomes accommodated to these animals and then after inoculation the organisms rapidly multiply in the blood, producing an acutely fatal disease; this infection can be sterilised by a variety of drugs, and cure often follows when a therapeutic agent is employed in a small fraction of the highest dose tolerated by the host. On the other hand, *T. gambiense* however long it is passed through mice continues as a rule to cause a more or less chronic and relapsing infection which is difficult to cure. There are many other examples in point, such as the curability by drugs of *T. brucei* infection in the rat and the resistance of *T. lewisi*. As regards the mechanism of cure, there is evidence that the effect of a drug which has an affinity for constituents of the trypanosomes, while accounted for partly by this direct action, may also be due in part to an immunity mechanism, since the parasites can act as antigens. Where, as in the above instances, the species of the host is the same, the differences in therapeutic action must be accounted for by variations in properties of the trypanosomes, namely (*a*) their susceptibility to the direct action of the drug, and (*b*) their capacity for exciting an immunity response. Alterations in either or both of these properties may occur. When comparing the behaviour of different species of trypanosomes it is difficult to attribute variations in therapeutic response definitely to one factor. Nevertheless, it is a striking fact that drug fastness should be manifested in the chronic and relapsing infections in which the resistance of the host is obviously well marked rather than in the acute disease where resistance is at a minimum.

[1] Work done with the support of the Medical Research Council.

The present observations indicate clearly that the response to treatment shown by a strain of trypanosomes may vary directly with the degree of pathogenicity which the parasites exhibit toward the host. A strain of *T. brucei*[1] was obtained from a dog which had been exposed to the bites of "wild fly" in Uganda and died within five weeks of their first feeding. Its blood after citration was injected into guinea-pigs and in the course of eight months the trypanosomes were passed through six of these animals by injections of blood. From the sixth guinea-pig mice were inoculated and thereafter the infection was passed solely through these animals. In order to accommodate the strain a passaage was made whenever parasites became abundant in the blood; at first 0·5 c.c. of a 6–8 per cent. suspension of blood in saline was injected subcutaneously and later 0·5 c.c. of a 2–3 per cent. suspension. Table I shows the

Table I

No. of passage ...	1	2	3	4	5	6	7	8	9	10	11	12	13	14–17	18
Duration of the infection†	17 (*2*)	* (*3*) 11 (*3*) 14 (*3*) 18 (*4*)	17 (*2*)	18 (*2*)	14 (*3*)	12 (*3*)	13 (*2*)	10 (*3*)	9 (*2*)	4 (*3*)	7 (*2*)	7 (*3*) 7 (*3*)	4 (*2*) 8 (*2*)	3–4 (*2–3*)	3 (*2*) 7 (*2*)

The numbers in italics indicate the incubation periods in days between inoculation and the appearance of scanty parasites in the blood.

* This mouse, from which the 3rd passage was derived, was treated afterwards.

† Days from first appearance of trypanosomes in the blood till death.

progress of accommodation of the trypanosomes to mice, the number of days elapsing between their first appearance in the blood and death from the infection being given. Up to the 12th passage most of the animals showed a marked fluctuation in the numbers of parasites in the blood before death finally took place, when they were numerous. This fluctuation, as well as the length of survival, are better indications of the state of accommodation of the trypanosomes than is the incubation period (also shown in Table I). From the 14th passage onward the strain possessed nearly maximal pathogenicity and the parasites on appearing in the blood increased progressively till death, which in all animals, except one in the 18th passage, occurred within three days thereafter. Accordingly, the strain accommodated itself fairly rapidly to the mouse and finally exhibited the high pathogenicity characteristic of *T. brucei*.

Therapeutic tests with various trypanocidal substances were carried out during the early passages (1–8) and also at a later stage (passages 34–290); the drugs were injected subcutaneously. The results (Table II) show that the infection at first was markedly resistant toward all the substances of various classes—arsacetin, tryparsamide, trypanblue, Bayer 205, trypaflavine, and styryl-quinoline compounds. Although the number of animals treated with each substance is not large the findings as a whole are striking. Of twenty mice infected with the unaccommodated parasites only three were cured with the range of doses used. On the other hand, with dosages which were equal or

[1] We are indebted for this to Dr A. Wormall, University of Leeds.

Table II. *Chemotherapeutic response*

	Early passages (1–8)			Late passages (34–290)		
Drug	Dose	No. of tryps. in blood	Result	Dose	No. of tryps. in blood	Result
Arsacetin	1:40	m	R, 7 day	1:50	vm	C
	1:40	f, *R*	R, 9 day			
	1:50	f	C			
Tryparsamide	1:150	f	R, 5 day	1:150	f	C
Trypanblue	1:200	f	R, 5 day	1:200	f	C
Bayer 205	1:1,000*	f	R, 43 day	1:3,000	f	C
	1:2,500	f, *R*	C	1:5,000	f	C
	1:5,000	f	R, 3 day	1:7,500	f	C
	1:10,000	f	R, 3 day	1:10,000	f	C
				1:10,000	f, *R*	C
				1:15,000	f	C
				1:15,000	f	R, 9 day
				1:15,000	f	R, 12 day
Trypaflavine	1:3,000	f	R, 7 day	1:3,000	f	C
				1:6,000	f	C
				1:7,500	f	C
Styryl 90†	1:1,000*	f	R, 19 day	1:3,000	f	C
	1:1,500	m, *R*	R, 25 day	1:5,000	f	C
	1:5,000	f	R, 8 day	1:7,500	f	R, 8 day
	1:10,000	f	R, 6 day	1:10,000	f	C
				1:10,000	f	C
				1:10,000	f	C
				1:15,000	f	R, 30 day
Styryl 314‡	1:1,500	f, *R*	R, 7 day	1:3,000	f	C
	1:2,500	f, *R*	C	1:5,000	f	C
	1:5,000	f	R, 6 day	1:7,500	f	R, 10 day
	1:7,500	f	R, 6 day	1:10,000	f	C
	1:10,000	f	R, 15 day	1:10,000	f	R, 7 day
Styryl 245§	1:500	f	R, 47 day	1:5,000	f	C

The dosage is at the rate of 1 c.c. of the concentration shown for a mouse weighing 20 g.

The number of trypanosomes present in the blood at the time of treatment is indicated as follows:

f = few—the number found as a rule 24 hours after the usual inoculation with the fully accommodated strain.

m = many—the number found 48 hours after the usual inoculation with the fully accommodated strain.

vm = very many—the number found 72 hours after the usual inoculation with the fully ac commodated strain.

The therapeutic response is indicated thus:

C = cure.

R = relapse; the number following shows the day after treatment on which parasites reappeared in the blood.

R indicates that treatment was given in a relapse following a smaller dose of the same substance.

* The animal had previously received an injection of human serum; control tests showed tha this had no significant influence on the chemotherapeutic response.

† Browning, Cohen, Ellingworth and Gulbransen (1929).

‡ The methosulphate corresponding to styryl 90, which is a methochloride.

§ Browning, Cohen, Cooper, Ellingworth and Gulbransen (1933).

smaller twenty-one were cured out of twenty-seven mice infected with the accommodated parasites. In detail the results with arsacetin, Bayer 205 and the styryl compounds are highly significant. Thus the unaccommodated trypanosomes resisted treatment with arsacetin 1:40 and 1:50 administered when only scanty or moderate numbers of parasites were present in the blood, although in a late passage 1:50 cured the infection at an advanced stage. Bayer 205 in a dose of 1:1000 and 1:5000 and also styryl 90 and styryl 314 in a dose of 1:1000 or 1:1500 also failed to effect cure when trypanosomes were scanty. These represent considerable multiples of the curative dose for the accommodated parasites. Now there is extensive evidence that the latter showed toward these drugs very much the same behaviour as several other strains of *T. brucei* which had been passed for long periods through mice.

These observations are paralleled by those of Kroó (1926) with *Spirochaeta recurrentis*. The strain of this spirochaete in its original state was highly virulent for mice on inoculation with infective blood and at the same time was highly sensitive to treatment with salvarsan. As a result of passage through ticks the virulence of the spirochaetes was diminished for the mouse in the sense that, following inoculation the infection developed after a prolonged incubation period, the parasites multiplied less quickly, the health of the infected animals did not suffer and the mortality was reduced. In addition, the attenuated strain had become resistant to the drug, since cure, *i.e.* absence of blood relapses, resulted in only one-sixth of the treated animals, as compared with cure in five-sixths of those inoculated with the virulent spirochaetes and treated with the same dose of salvarsan. It is noteworthy also that according to Bang, Madsen and Mörch (1928) rabbits inoculated with a virulent strain of *Bacillus tuberculosis* were more readily cured with sanocrysin than those infected with a less virulent strain.

SUMMARY AND CONCLUSIONS

A strain of *Trypanosoma brucei* when recently introduced into mice and imperfectly accommodated to this species of host produced infections relatively resistant to various trypanocidal drugs. On the other hand, when the strain had become highly accommodated and its pathogenicity increased to a maximum as the result of repeated passages, infected animals were readily cured. The exact mechanism on which this difference in resistance may depend has not been investigated. Attention is directed, however, to the important fact that the drugs acted poorly when the strain was in an attenuated state, as shown by the prolonged course of infection and the occurrence of marked fluctuations in the number of parasites present in the blood. Thus the chemotherapeutic response was weak at the time when the host itself was able to exercise an effective resistance. Later, when the host's resistance had become negligible, the curative action of the drugs was pronounced.

REFERENCES

BANG, O., MADSEN, TH. and MÖRCH, J. R. (1928). *Z. Hyg. InfektKr.* **109**, 243.

BROWNING, C. H., COHEN, J. B., COOPER, K. E., ELLINGWORTH, S. and GULBRANSEN, R. (1933). *Proc. Roy. Soc.* B, **113**, 300.

BROWNING, C. H., COHEN, J. B., ELLINGWORTH, S. and GULBRANSEN, R. (1929). *Ibid.* B, **105**, 99.

KROÓ, H. (1926). *Deutsche med. Wochenschr.* **52**, 1032.

NOTE

Professor Warrington Yorke (personal communication) has found that strains of *T. rhodesiense* which had been repeatedly passed through mice were more susceptible to arsenicals than those which had not. This increase in susceptibility seemed to occur broadly with increase in virulence.

(*MS. received for publication* 5. II. 1935.—Ed.)