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Persistence of the Parabasal Body in a p-Rosaniline Resistant Strain of *Trypanosoma brucei.*

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It has been known for a long time (Werbitzki[1] that certain anti-trypanosomal drugs, *e. g.*, Pyronines, Acridines, Triphenylmethane dyestuffs, elicit a very definite response of the parabasal body of trypanosomes to the effect that this structure can no longer be demonstrated by the usual staining methods such as Wright's, Romanowsky's and Giemsa's stain. It is, moreover, known that trypanosome strains rendered resistant to these drugs may retain the lack of the parabasal body for many years. It was, therefore, surprising to note that in a strain of *T. brucei* which was made resistant to *p*-rosaniline hydrochloride the parabasal body persisted after the parasites had reached maximal resistance to this dyestuff. This rather unique observation seems interesting enough to justify a short description of the preparation and the properties of this drug resistant strain.

The strain of *T. brucei* was kindly given to us by Dr. M. Soule. According to a recent paper by Merchant[2] and a personal communication by Dr. Soule the strain was derived from an original isolation by Bruce (1896) and was kept in guinea pig passages for about 40 years. We became interested in these trypanosomes when it was observed[3] that the organisms possessed a higher sensitivity towards *p*-rosaniline hydrochloride. It is well known that this triphenylmethane dyestuff is of very low activity. Most of the commonly used laboratory strains of *T. equiperdum*,[4] *T. brucei, T. rhodesiense* require a subcutaneous treatment with the maximal tolerated dose (50 mg/kg) or an oral administration of 250 to 500 mg/kg in order to clear the peripheral blood for a period of 3 to 6 days. No permanent cure of the infection can be obtained at least not with a single administration of the dyestuff. An infection of mice with the present *T. brucei* strain responded with permanent disappearance of trypanosomes to a single subcutaneous dose of 50 mg/kg or a single oral dose of 250 mg/kg. Smaller doses down to 10 mg/kg subcutaneously or 100 mg/kg orally cleared the peripheral blood for a 4-6 days period after which relapses occurred. Only doses of 5 mg/kg and 10 mg/kg given by the subcutaneous and oral route respectively failed to show any significant activity.

Drug resistance of this strain towards *p*-rosaniline hydrochloride was obtained by the so-called "short-passage method" described in an earlier paper.[4] Starting with an oral dose of 10 mg/kg the strain became resistant to 500 mg/kg *per os* after 25 to 30 passages. The treatment with this dose of *p*-rosaniline was continued to a total of 47 passages in order to consolidate the fastness. The strain was eventually resistant to 500-1000 mg/kg *per os*. The dose of 1000 mg/kg which is close to the maximal tolerated dose still produced occasionally a more or less marked decrease in the trypanosome content of the peripheral blood, but never cured the animals.

Early in the process of rendering the strain resistant it was noted that the parabasal body persisted in the parasites. Despite the continued administration of *p*-rosaniline and the corresponding increase of resistance towards the dyestuff the parabasal body retained its tinctorial affinity and was easily demonstrated in smears from Giemsa's stain. This is shown in Fig. 1.

This persistence of the parabasal body in *p*-rosaniline-resistant trypanosomes has to our

[1] Werbitzki, F. W., *Centralbl. f. Bakteriol.*, 1910 I Orig., 1910, **53**, 303.

[2] Merchant, D. J., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 391.

[3] Ziering, A., and Buck, M., *E. Ch. Barell*, Jubilee Volume, Basle, 1946, p. 378.

[4] Schnitzer, R. J., Lafferty, L. C., and Buck, M., *J. Immunol.*, 1946, **54**, 47.

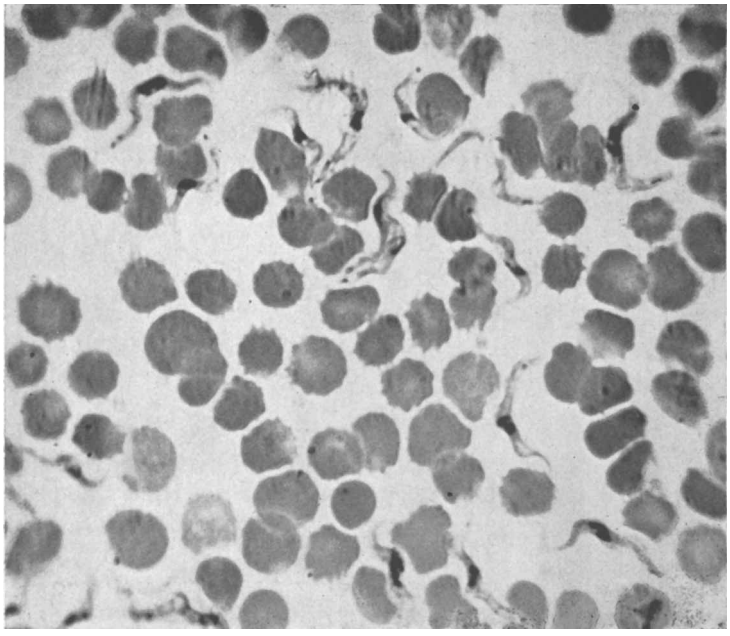

FIG. 1.
Blood of a mouse infected with the *p*-rosaniline resistant strain of *T. brucei*. (47th passage.) Giemsa's stain. Objective apochromate HE 90, compens. eye piece 10 ×. (Photomicrograph by L. Hodax.)

knowledge not been described before. In a number of trypanosome strains rendered resistant to the dyestuff in our laboratory a similar phenomenon was never observed. In particular, of 9 *p*-rosaniline-fast strains of *T. equiperdum* which were prepared in mice and rats and studied during recent years none failed to show the absence of the parabasal body in 80-100% of the individual parasites.

The unusual observation in the resistant strain of *T. brucei* made it desirable to examine the properties of this strain more closely and particularly to investigate its response to different anti-trypanosomal agents.

The results of these specificity tests of the resistant strain are given in Table I.

Mice infected with the parent strain and the *p*-rosaniline-fast trypanosomes were treated with a group of different chemotherapeutic agents of known activity such as 3,3'-4,4'-dihydroxy arseno-benzene-N-methanal sulfoxylate (Neoarsphenamine), 3-amino-4-hydroxyphenyl arsineoxide hydrochloride (Mapharsen), potassium antimonyl tartrate (Emetic tartar), 2,8-diaminoacridinium-10-methochloride (Acriflavin), and a quinoline compound, 6,6'-ureylene bis[4-amino-2-methyl quinoline].

From the data of the minimal active doses, as given in the table, it is evident that the resistance was specific for *p*-rosaniline; the arsenicals, the antimony compound, and the acridine dyestuff showed the same activity on the resistant as on the parent strain. That is characteristic for *p*-rosaniline-fast trypanosome strains.

The observation that the quinoline compound was also active in the infection with the *p*-rosaniline-fast strain was surprising. This compound is a member of a large series of anti-trypanosomal agents[5] and according to previous experience (Schnitzer, unpublished data) proved to be active in experimental infections with normal trypanosomes but without effect on the *p*-rosaniline-resistant modifications of these strains. For instance: Two

[5] Jensch, H., *Angewandte Chemie*, 1937, **50**, 891.

TABLE I.
Sensitivity of the Normal and the *p*-Rosaniline Fast Strain of *T. brucei* Toward Different Anti Trypanosomal Agents.

Compound	Route	Minimal active dose (mg/kg)	
		Normal strain	Resistant strain
Neo-arsphenamine	subcut.	12.5	12.5
Mapharsen	"	1.0	1.0
Acriflavine	"	5.0	5.0
Emetic tartar	"	5.0	5.0
6,6′-ureylene-bis (4-amino-2-methyl-quinoline)	"	62.5*	62.5*
p-Rosaniline HCl	"	10.0	> 50.0
	per os	100.0	>500-1000.0

* Identical with the curative dose.

p-rosaniline-fast strains of *T. equiperdum** (PR I, PR III) which also lacked the parabasal body no longer responded to subcutaneous treatment with 500 mg of the quinoline derivative per kg, while a tenth of this dose (50 mg/kg) cured the infection with the parent strain.

These observations indicate that an atypical *p*-rosaniline-fast strain of *T. brucei* was obtained. It was characterized by the persistence of the parabasal body and the therapeutic sensitivity to 6,6′-ureylene bis (4-amino-2-methylquinoline). *p*-Rosaniline-resistant modifications of *T. equiperdum* exhibited as a rule the loss of both these properties. The question of the mechanism by which these 2 phenomena, namely, presence or absence of the parabasal body and sensitivity towards 6,6′-ureylene bis [4-amino-2-methyl quinoline] are related or whether they are related at all cannot be decided on the basis of our present experience. We are also not in a position to state definitely that other strains of *T. brucei* might give a similar response to *p*-rosaniline or even that the same strain of *T. brucei* will always react in the same atypical way to attempts of rendering it *p*-rosaniline-fast.

Summary. A *p*-rosaniline-resistant modification of *T. brucei* is described in which—contrary to the experience with other trypanosome strains—the parabasal body persisted. The strain also failed to show the overlapping resistance to 6,6′-ureylene [4-amino-2-methylquinoline] which could be demonstrated in *p*-rosaniline-fast strains of *T. equiperdum.*

* The experiments with *T. equiperdum* were carried out by Schnietzer in the Connaught Medical Research Laboratories of the University of Toronto.

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Stability of Natural Progesterone.*

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In 1936 approximately one gram of crystalline progesterone was isolated from sows' ovaries for use in the study of some of the biological properties of the hormone.[1] The major portion of this lot was used in the study of various aspects of the hormonal control of

* The authors wish to thank Miss Lila F. Knudsen, of the Food and Drug Administration, Washington, D.C., for her assistance in the statistical analysis of the data.

[1] Allen, W. M., and Goetsch, Carl, *J. Biol. Chem.*, 1936, **116**, 653.