Akinetoplastic Strain of *Trypanosoma evansi* produced by 'Prothidium'

SINCE Werbitzki's¹ observations that organic dyes having Ehrlich's orthoquinoid constitution caused disappearance of the kinetoplast in trypanosomes, several workers have, from time to time, reported the occurrence of both spontaneous and chemically induced strains of akinetoplastic *T. evansi*. Hoare² has fully discussed the early literature on the subject. Recently, Muhlfordt³ has published his observations on the effect of trypanflavin on the kinetoplast of various trypanosomes, and has shown that repeated administration of trypanflavin caused the complete disappearance of kinetoplast in *T. evansi* in 81 days. With other trypanosomes such as *T. equiperdum*, *T. brucei* and *T. gambiense* it took 54, 121 and 122 days, respectively, for the complete disappearance of this organelle.

Hoare and Bennett⁴ in 1938 showed that both spontaneous and induced akinetoplastic strains of *T. evansi* behaved like the normal ones, and that the absence of kinetoplast did not in any way alter the vital functions of the trypanosomes.

We have observed: (1) the quick disappearance of the kinetoplast from the otherwise normal strain of *T. evansi* as a result of treatment with a phenanthridinium compound 'Prothidium'; and (2) some apparent change in the functional activity of the akinetoplastic 'Prothidium'-treated strain.

The chemical composition of 'Prothidium' (Boots) is 2-amino-7-(2-amino-6-methyl-4-pyrimidylamino)-9-amino-phenyl-phenanthridine: 10:1-dimethoxy-bromide. 0.5 ml. of a freshly made 0.015 per cent solution of this drug was given subcutaneously to white rats weighing 100–125 gm. and showing a fair number of trypanosomes in the peripheral circulation. Three sets of experiments were carried out with two rats in each set. The ratio of kinetoplastic to akinetoplastic trypanosomes was estimated by counting a hundred trypanosomes in a stained smear made from one of the rats in each series. The akinetoplastic 'Prothidium'-treated strain is now being maintained in this laboratory and its functional behaviour so far observed (up to its 8th passage in rats) is reported as follows.

From Table 1 it will be seen that there was complete disappearance of the kinetoplast 44–60 hr. after treatment with the drug.

Table 1. PERCENTAGE OF KINETOPLASTIC (K) AND AKINETOPLASTIC (AK) TRYPA NOSOMES AT DIFFERENT HOURS

	18 hr.	44 hr.	60 hr.
Series I	K.28 : AK 72	All akinetoplastic	
Series II	K.42 : AK 58	K.17 : AK 83	
Series III	K.34 : AK 66	K.20 : AK 80	All akinetoplastic trypanosomes disappeared*

* There was a relapse after 30 days when only AK forms were seen.

The akinetoplastic strain thus obtained was found to breed true when passaged through rats. Table 2 shows an alteration in the functional activity of the akinetoplastic 'Prothidium'-treated strain as compared to the normal.

From Table 2 it is apparent that the akinetoplastic trypanosomes multiply less rapidly than the normal strain, and this leads to longer survival after infection. Ultimately, however, the host succumbs to infection with the same clinical and pathological picture as seen in rats infected with a normal strain of *T. evansi*. This

Table 2. PREPATENT AND SURVIVAL PERIODS OF RATS INFECTED WITH NORMAL AND 'PROTHIDIUM'-TREATED STRAIN OF *T. evansi*

	Normal strain	'Prothidium'-treated strain
Incubation period in days in six rats	2, 2, 2, 2, 3, 3	5, 5, 6, 9, 12, 12
Survival time in days after first appearance of trypanosomes	3, 3, 3, 4, 3, 4	3, 5, 8, 9, 12, 12

akinetoplastic strain is now in its 8th passage, and the results are awaited with regard to its course of infection in larger animals (dog and horse).

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¹ Werbitzki, F. W., *Centralbl. Bakt., Abt. 1. Orig.*, **53**, 303 (1910).

² Hoare, C. A., *J. Protozool.*, **1**, 28 (1954).

³ Muhlfordt, H., *Z. Tropenmed. u. Parasit.*, **10**, 19 (1959).

⁴ Hoare, C. A., and Bennett, S. C. J., *Parasitol.*, **30**, 529 (1938).

Studies on Phycobilin Formation with Mutants of *Cyanidium caldarium*

VARIOUSLY pigmented mutants of *Cyanidium caldarium*, an organism classified in succession as a blue-green, green, and red alga by Hirose¹ and which Allen² regards as an anomalously pigmented chlorophyte, appear to be excellent experimental materials for study of the synthesis of phycocyanin.

Pigment analysis of light-grown wild-type *C. caldarium* confirmed Allen's² report of the presence of chlorophyll *a*, carotenoid, and phycocyanin in this organism. In addition, allophycocyanin was found to be present. Absorption spectra of the extracted pigments are shown in Fig. 1. Dark-grown wild-type cells, unlike those reported by Allen², have been found to yield measurable amounts of chlorophyll *a* as well as carotenoid. As in the work reported by Allen² no phycobilins have been found in dark-grown wild-type cells. Mutant forms were obtained by exposure of wild-type or mutant cells to ultraviolet light. Absorption spectra of whole-cell suspensions of the wild and mutant types are shown in Fig. 2. Solutions of individual pigments were obtained as follows: (a) Cells were broken by grinding with polishing alumina or by means of a 10 kc./s. Raytheon sonic oscillator. (b) The chlorophyll and carotenoid pigments were then extracted in methanol and petroleum ether and separated on a sugar column developed with petroleum ether and *n*-propanol after the method described by Strain³. (c) The water-soluble phycobilins were separated on a calcium phosphate column⁴ developed with 0.06 *M* sodium phosphate. Absorption spectra of extracted pigments and whole-cell suspensions were determined with a Cary recording spectrophotometer, model No. 11. The opal glass procedure of Shibata *et al.*⁵ was used for the determination of absorption spectra of suspensions of intact cells. The organisms were grown at room temperature or at 43°C. on a medium similar to that used by Allen², to which 1 per cent glucose was added.

Four general types of variant forms have been found.

(1) *Dark-greening mutants*. Cultures of wild-type cells grown in darkness are yellow; carotenoid and traces of chlorophyll are detectable spectrophoto-