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In vitro Photodynamic Action of Methylene Blue on *Trypanosoma brucei.*

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In reviewing the literature dealing with the *in vitro* photodynamic action of acridine dyes on microörganisms, one is impressed with the fact that adequate study of this action on protozoa has yet to be made. The few reports in existence deal almost exclusively with the effect of the photodynamic action of various dyes on the motility of a few protozoa. Such were the studies made with *Leishmania donovani* flagellates by Noguchi[1] and those of trypalavine on *Trypanosoma brucei* by Tappeiner,[2] Bruynoghi,[3] and Jancso.[4] It seems plausible that motility alone cannot be taken as the criterion of life or death of the treated organisms. In addition, it is not inconceivable that such action might bring about some morphological changes on the treated protozoa. With these views in mind, it was considered of interest to restudy the *in vitro* photodynamic action on *Trypanosoma brucei* with methylene blue, a dye which hitherto has not been tried on this organism.

Citrated blood of guinea pigs infected with *Trypanosoma brucei* was diluted with normal saline. After the cells had been removed by centrifugation at low speed, the supernatant fluid which contained the organisms was mixed with various dilutions of saturated solution of methylene blue in the ratio of 9 to 1 respectively. Part of the mixture contained in a Petri dish was put over a Frigidaire cooling machine and exposed to a 100-Watt lamp at a distance of 10 cm. for one hour. The other portion of the mixture was kept unexposed as control. The motility of the exposed and unexposed *Trypanosoma brucei* was examined at the end of 30 and 60 minutes respectively. After exposing for one hour, 0.5 cc. of each of the exposed and unexposed mixtures were injected intraperitoneally into a series of white mice, 4 in each group. Daily examination for the presence of trypanosomes in the blood of the inoculated animals was

[1] Noguchi, H., *Proc. International Conference on Health Problems in Tropical America, Kingston, Jamaica,* 1924, p. 455.

[2] von Tappeiner, H., *Erg. Physiol.*, 1909, **8**, 698.

[3] Bruynoche, par R., *Compt. rend. Soc. de Biol.*, 1932, **110**, 124.

[4] von Jancso, N., *Zentralb. f. Bakt.*, Original 1931, **122**, 388; 1932, **123**, 129.

made for 3 weeks. Part of the mixtures was kept at 37°C. and was examined from time to time to observe any change in morphology as stained with the Wright stain.

The following results were obtained: (a) Methylene blue, up to dilution of 1:100,000, in the presence of light, was sufficient to immobilize *Trypanosoma brucei,* whereas, without light this dye could only exert a similar effect in a concentration of 1:100. (b) Sixty minutes of exposure yielded results similar to a 30-minute exposure. (c) The immobilizing effect of the dye was parallel to its lethal activity. (d) All the mixtures containing immobilized trypanosomes failed to produce infection in white mice. Furthermore animals infected with trypanosomes treated with the photodynamic action of methylene blue in a dilution insufficient to immobilize the organisms were found to have a longer incubation period and survival than the control ones.

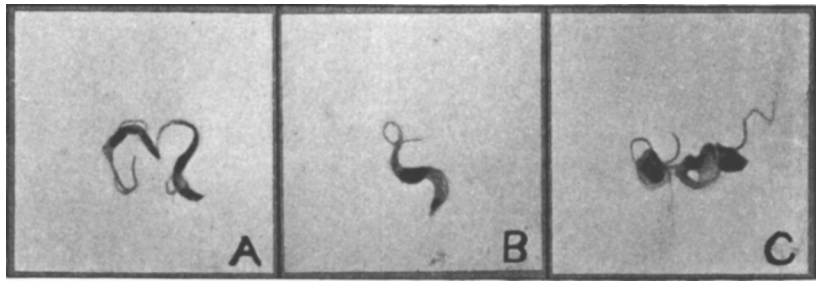

FIG. 1.
Morphological appearance of *Trypanosoma brucei* one week after treatment with various methods.
A. Inactivated by the photodynamic action of methylene blue.
B. Treated with methylene blue alone.
C. Preserved with 0.4% formalin.

As it has been previously shown that the photodynamic action of methylene blue exerts a preservative effect on pneumococci[5] the same preservative effect on the *Trypanosoma brucei* has also been demonstrated. As examined in stained smears, the cellular structure of the photodynamically treated trypanosomes—concentration of 1:100 or 1:1,000 of methylene blue kept at 37°C. for a month was used—is almost comparable to that of the fresh organisms. On the other hand, trypanosomes heated at 56°C. for half an hour lose their cellular integrity. When they were preserved with 0.4% formalin their shape became distorted and they began to show signs of disintegration if kept at 37°C. for 2 weeks. It is also worthy of

[5] T'ung, T., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 328.

note that *Trypanosoma brucei,* although equally susceptible to the photodynamic action of eosin, cannot be preserved by this treatment.

The fact that *Trypanosoma brucei* can be well preserved morphologically by the photodynamic action of methylene blue even at incubator temperature for a considerable length of time, and that a large quantity of such treated organisms can be easily obtained by the method described, offers the possibility of studying active immunization in susceptible animals with a vaccine. Since the protective property of heat-treated trypanosoma vaccine has not been satisfactorily demonstrated such a method is worthy of further investigation.

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Observations on the "Latent Period" in Experimental Diabetes Insipidus.*

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The "latent period" as designated and described by Fisher, Ingram, and Ranson[1] was encountered by us without exception in upward of 30 experiments in the dog where permanent d.i. of varying severity has followed hypothalamic lesions. The operation was usually followed immediately by a polydipsia and polyuria which was invariably interrupted by a return of the water exchange toward normal, usually reaching to below normal, in a matter of 2 to 6 days. The more or less abrupt onset of the permanent phase of polydipsia and polyuria occurred on the seventh to eleventh postoperative day. Our series differed from the Fisher series in that the d.i. of the temporary phase was, as a rule, greater in magnitude than that of the permanent phase.

In view of the evidence indicating that a functioning pars anterior of the hypophysis is essential for the occurrence of d.i. *in its maximal form,* it seemed possible that the events of the latent period might be explained on the basis of the operative procedure produc-

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1 Fisher, C., Ingram, W. R., and Ranson, S. W., *Arch. Neurol. and Psychiat.,* 1935, **34**, 124.