most rapidly. In our study, the mercurial was injected far more slowly, from 0.4 to 1.6 mg Hg per kg per minute. Values approximating ours for the lethal dose of mercupurin, mercurin, salyrgan, and salyrgan-theophylline were obtained by DeGraff and Lehman,[1] when they injected the mercurial slowly (1 mg Hg per kg per minute). This favors the conclusion that in their titrations the actual acute lethal dose was overrun, in the case of their rapid injections, in proportion to the speed of administration.

Conclusions. (1) The admixture of theophylline with salyrgan reduces the toxicity of the latter as the result of some reaction between the two compounds inasmuch as aging is required before the changed toxicity is demonstrable. (2) This influence of theophylline did not apply to mercurin. (3) In terms of mercury, non-ionizable organic mercurials are not necessarily less toxic than mercuric chloride.

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Cultivation of *Trypanosoma gambiense in vitro* in Cell-Free Medium.*

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The diagnosis of African Sleeping Sickness has depended upon confirming clinical impressions by a direct demonstration of trypanosomes in the blood, lymph node fluid, or spinal fluid of the patient or else indirectly by animal inoculation. It seems that many infected cases might be overlooked by these methods, a particularly unfortunate circumstance in the early stages of the disease when treatment is usually so very effective.

In this preliminary paper it will be shown that it is easy to cultivate *Trypanosoma gambiense*, and that by this means infection may be demonstrated when other methods fail. Amongst previous investigators of this problem there is space here to mention only Brutsaert and Henrard[1] whose brilliant and successful work with cultures at Leopoldville, Belgian Congo, was a marked stimulus.

Two strains of *Trypanosoma gambiense*, both originating in Liberia, were used.† Both behave similarly in cultures.

The culture medium has the following formula: Part a: Sodium chloride 8 g, nutrient agar 1.5% (Difco) 4 g, distilled water, to make 900 cc; Part b: Human plasma (citrated) 100 cc, human hemoglobin 20 cc.‡

The base (part a) is sterilized in the autoclave, and part b added later. The medium is dispensed in test tubes and corked with rubber stoppers. The final pH is 7.4-7.5. It will be seen that the medium is a modification of that used by Noguchi and Battistini[2] for the cultivation of *Bartonella bacilliformis*.

Cultures are incubated at room temperature (26-28°C), and become positive 7 to 10 days after inoculation, even when the original inoculum contains no microscopically demonstrable trypanosomes, but the tubes should not be discarded until after one mon'h. The cultures remain viable for 60 days or more, the maximum thus far observed being 71 days. After 2 weeks of cultivation, the pH of the medium drops to 7.2.

Thus far isolations from inoculated susceptible animals have been regularly successful.

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1 Brutsaert, P., and Henrard, C., *C. R. Soc. de Biol.*, 1938, **127**, 1469.

† Obtained through the kindness of Dr. Henry D. Murray and Dr. G. G. Campbell.

‡ Made by laking 1 part of blood with 3 parts of distilled water.

2 Noguchi, H., and Battistini, T., *J. Exp. Med.*, 1926, **43**, 851.

This result was obtained when the donors were in a "negative period" and indeed even when no trypanosomes had ever been microscopically demonstrable. Thus *M. mulatta* 43104, inoculated September 3, became infected. On September 29 and October 4 trypanosomes were seen, and on each of those days blood of this monkey was inoculated by the intraperitoneal route into rat 43111F. The trypanosomes were never found in the blood of this animal in 29 daily examinations from October 4 to November 2. However, cultures made on October 18 became positive and proved that the rat had become infected.

That the cultural method is more sensitive than the animal inoculation test for detecting light infections is also indicated by the following: Monkey 43104 did not react positively following the inoculation of a killed trypanosomal antigen on October 12 at which time *T. gambiense* was no longer demonstrable in the blood. The question arose whether this result could be attributed to eradication of the infection. On October 21 the animal was bled and two 140-g rats (43119) received respectively 1.0 cc and 0.5 cc of the blood intraperitoneally. During one month and a half no trypanosomes were found in the blood of either animal. Conversely, cultures inoculated with 0.3 cc of the same blood were positive in 10 days.

Cultures have now been maintained through numerous transfers for a period of 127 days. Following each transfer multiplication takes place with the production of numerous dividing forms so that unlimited survival *in vitro* seems not unlikely.

Multiplication takes place by the longitudinal division of trypanosome forms into 2 or more daughter individuals. Rosettes or clumps of trypanosomes are frequent. Quite common are very slender organisms with a narrow undulating membrane measuring, after staining, only 1.5-2.0 μ in greatest width; these appear to correspond to the proventricular forms seen in the vector (*Glossina*). Another form is easily twice this diameter, possesses a wide conspicuous undulating membrane, and there are various intermediaries between these two. Multiple division forms composed of from 3 to 6 or more individuals also resemble forms described in Glossina. Another peculiar type are trypanosomes with an enlarged globular posterior extremity.

Summary. 1. *Trypanosoma gambiense* is readily cultivated on a semi-solid medium containing human plasma and hemoglobin. 2. Infection can be detected by cultures, when the usual methods of examination and inoculation of the blood fail. 3. Successful numerous transfers, each followed by multiplication, suggest that the cultures may be able to be maintained indefinitely.