hours while the total urinary excretion of the compound over a period of 24 hours following its administration varied from 22 to 48% of the amount administered.

The serum levels in 3 additional adult subjects (No. 4, 5 and 6) following single intramuscular injections of 0.25 g (250,000 units) of streptomycin along with the oral administration of sodium benzoate in dose of 10 grains (0.65 g) for 6 doses totalling 60 grains (3.9 g) in subject No. 4 and 20 grains (1.3 g) for 6 doses totalling 120 grains (7.8 g) in subjects No. 5 and 6 were practically the same at the end of 6 hours, being 1.0, 2.5 and 2.5 units per cc, respectively. Furthermore, the total urinary excretion of streptomycin in these subjects was actually higher than observed in the 3 given streptomycin alone, having varied from 36 to as much as 75% of the dose of streptomycin administered.

In an additional experiment, 0.5 g (500,000 units) of streptomycin were given to each of 2 adult subjects (No. 7 and 8) by intramuscular injection. One of these (No. 7) served as a control while the second (No. 8) was given 20 grains (1.3 g) of sodium benzoate every 4 hours totalling 120 grains (7.8 g) in the period of 24 hours. The results were similar in that the serum level at the end of 6 hours (2 units per cc) in subject No. 8 receiving sodium benzoate was practically the same (2.5 units per cc) as observed in the control. In this experiment, however, while the control (No. 7) showed a total urinary excretion of 44% of the dose of streptomycin administered, subject No. 8 receiving sodium benzoate showed a total urinary excretion of only 30% of the dose of streptomycin injected, while the serum levels at the end of 2, 3 and 4 hours after injection were slightly higher than were observed in the control.

Summary. The results indicate, therefore, that the oral administration of sodium benzoate in tablets in dose of 10 to 20 grains (0.65 to 1.3 g) every 4 hours for 6 doses totalling 60 to 120 grains (3.9 to 7.8 g) along with single intramuscular injections of 0.25 and 0.5 g (250,000 and 500,000 units) of streptomycin does not appear to reduce materially the urinary excretion of streptomycin, and does not enhance or prolong the maintenance of the serum levels of the compound. It is to be emphasized, however, that these preliminary results are inconclusive and, in view of the clinical importance of the subject in relation to the administration of streptomycin, additional investigations are being conducted.

15635 P

Cultivation of African Sleeping Sickness Trypanosomes on Improved, Simple, Cell-Free Medium.*

DAVID WEINMAN.

From the School of Public Health, Faculty of Medicine, Columbia University.

Progress in the investigation of many important aspects of African trypanosomiasis should be appreciably facilitated, it would seem, were it possible to grow the causative organism readily and in large quantities *in vitro*. Following the cultivation of the closely related *Trypanosoma brucei* by Novy and McNeal,[1] which acted as a notable stimulus, possible cultural methods for the human trypanosomes were investigated by Behrens,[2] Ponselle,[3] von Razgha,[4] Reichenow[5] and oth-

* It is a pleasure to acknowledge the aid received from Harvard University and The American Foundation for Tropical Medicine, Inc., joint sponsors of the 1944 Harvard Expedition to Liberia, during which the author obtained the strains of trypanosomes studied.

[1] Novy, F. G., and McNeal, W. J., *J. Inf. Dis.*, 1904, **4**, 1.

[2] Behrens, C. A., *J. Inf. Dis.*, 1914, **15**, 24.

[3] Ponselle, A., *C. R. Acad. Sci.*, 1924, **178**, 1219.

[4] von Razgha, A., *Z. f. Parasitenkunde*, 1929, **2**, 55.

[5] Reichenow, E., *Z. f. Parasitenkunde*, 1932, **4**, 784.

ers. Since none of the methods devised was completely satisfactory, improvements were sought by Brutsaert and Henrard[6] and, more recently, by Weinman,[7] with a semisolid agar. This last permits of indefinite serial transfers and is satisfactory for certain, but not for all purposes; accordingly, experiments were continued until the medium herein reported was developed.

This medium, which is suitable for the cultivation of both *T. gambiense* and *T. rhodesiense*, appears to have advantages over those previously described. Its favorable features are that growth is sufficiently heavy so that the trypanosomes can be obtained in large quantities and in almost any concentration desired, that the organisms thus harvested may be washed free of culture medium, that the growth may be initiated from small inocula of blood, and that the medium is relatively simple, easy to prepare, and stable, so that large quantities may be made prior to use and stored.

The medium is essentially a solid blood agar, containing inactivated, citrated, human plasma. The formula is as follows:

1. Base (autoclaved portion)

Nutrient agar (Difco 1.5%,† pH 7.3)	31 g
Distilled water, to make	1000 cc

For approximately 100 cc of medium, take 75 cc of the base and complete as follows:

2. Nonautoclaved portion

Human citrated plasma, inactivated‡	12.5 cc	
Human red cells	12.5 cc	
		25 cc
Base		75 cc

[6] Brutsaert, P., and Henrard, C., *C. R. Soc. Biol.*, 1938, **127**, 1469.

[7] Weinman, D., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 82.

† Manufacturer's formula for Difco nutrient agar 1.5%:

Beef extract	3 g
Bacto peptone	5 g
Sodium chloride	8 g
Agar	15 g
	31 g

To be dissolved in 1 L. distilled water.

‡ At 56°C for 30 min.

The medium can be dispensed in Kolle flasks or slanted in test tubes, and if stoppered with rubber corks or cotton covered with Parafilm to retard drying, may be stored in the refrigerator (5-8°C) for long periods.

Inoculated flasks are also stoppered to prevent evaporation and incubated at room temperature. Growth can be detected macroscopically by the development of small, rounded, colorless, transparent, slightly raised, glistening, moist-appearing areas, spotting the surface of the medium. These are colonies. They adhere to the surface of the agar, measure usually about 1-2 mm in diameter, more rarely up to 4 mm, do not hemolyze nor discolor the medium, and are composed of hundreds, or even thousands, of motile trypanosomes.

The numbers of trypanosomes inoculated determine the time required for the cultures to develop. If blood from the donor animal is diluted to contain 1,000 to 10,000 trypanosomes, growth is detectable in 5 days, and is heavy in 10; whereas if the inoculum contains 10 to 100 organisms, the cultures are often negative at 5 and positive at 10 days. Exceptionally, 3 or 4 weeks may be required before development is evident.

The period of heavy growth is usually maintained for about 10 days, then the trypanosomes gradually become less numerous, are quite scanty at the end of 35 to 40 days, but may persist for more than 60 days. Maintenance of the cultures by serial transfer presents thus no special problem. It becomes even simpler if low temperature facilities are available, Weinman and McAllister[8] having shown that cultures may be frozen and stored for long periods at —70°C.

A single harvest from one flask at or near the peak growth yields 10,000,000 to 15,000,000 trypanosomes. The organisms can be washed off in Tyrode's solution, and then concentrated by centrifuging. Washing and centrifuging may be repeated, so that ultimately one obtains a closely packed sediment consisting of trypanosomes surmounted by a water-clear liquid. As any number of

[8] Weinman, D., and McAllister, J., *Am. J. Hyg.*, in press.

flasks may be harvested at one time, and the yield combined, the number and concentration of trypanosomes can be adjusted within wide limits.

The multiplication in culture is massive and from an inoculum of 100 trypanosomes, some 30,000,000 may be obtained in a few weeks. This suggests that indefinite serial transfer will be possible on this medium, as it was on an earlier one,[7] which the author now considers inferior. However, since the strains have been maintained through only 10 generations *in vitro* the matter is not yet certain. Studies on the morphology and properties of the cultural trypanosomes will be reported shortly.

Conclusion. A blood-agar cell-free medium of relatively simple composition is described, which is suitable for the isolation and growth of both *Trypanosoma gambiense* and *Trypanosoma rhodesiense,* and appears to have advantages over others previously described.

15636

Effect of Diethylstilbestrol upon the Systolic Blood Pressure of Normal Rats.

HENRY C. HILL, JR. (Introduced by K. S. Grimson.)

From the Department of Surgery, Duke University School of Medicine, Durham, N.C.

The effect of diethystilbestrol upon the systolic blood pressure of rats has been variously reported. Grollman, Harrison and Williams[1] described hypertension during continuous oral administration in normal rats. Leathem and Drill[2] employed intramuscular injection in normal and in hypophysectomized rats and observed hypertension. Recently Oster[3] used oral administration in castrated rats and likewise demonstrated upward trends of blood pressures. Matthews, Emery, and Weygandt,[4] on the other hand, failed to obtain significant trends toward elevation of systolic blood pressure in normal or in castrate rats after prolonged oral administration during periods of treatment up to 100 days. These varied reports prompted a reinvestigation of the effect of diethylstilbestrol upon the blood pressure of normal rats. A preliminary appraisal of the present findings has been reported.[5]

Method. Thirty normal adult male and female albino rats of the Vanderbilt strain weighing between 180 and 360 g were used. They were maintained on Purina Dog Chow and water throughout the experiment. Systolic blood pressure was determined without anesthesia by the indirect tail plethysmographic method of Williams, Harrison, and Grollman[6] except that a 26 mm sphygmomanometer cuff, as recommended by Kempf and Page,[7] was employed. Water introduced into the plethysmograph was heated to body temperature. Systolic blood pressure was followed in 3 groups of rats:

I. Five male and 5 female rats received daily intramuscular injections of 1.0 mg of diethylstilbestrol in 0.2 cc of cottonseed oil for 24 successive days.

II. Five male and 5 female rats received daily injections of 0.2 cc of cottonseed oil for 24 days.

III. Five male and 5 female rats received

[1] Grollman, A., Harrison, T. R., and Williams, J. R., Jr., *J. Pharmacol. and Exp. Therap.*, 1940, **69**, 149.

[2] Leathem, J. H., and Drill, V. A., *Am. J. Physiol.*, 1943, **139**, 17.

[3] Oster, K. A., *Exp. Med. and Surg.*, 1946, **4**, 20.

[4] Matthews, C. S., Emery, F. E., and Weygandt, P. L., *Endocrinology*, 1943, **33**, 177.

[5] Hill, H. C., Jr., *Fed. Proc.*, 1946, **5**, 46.

[6] Williams, J. R., Jr., Harrison, T. R., and Grollman, A., *J. Clin. Invest.*, 1939, **18**, 373.

[7] Kempf, G. F., and Page, I. H., *J. Lab. and Clin. Med.*, 1942, **27**, 1192.