

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.

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

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The effect of diethylstilbestrol upon the systolic blood pressure of rats has been variously reported. Grollman, Harrison and Williams¹ described hypertension during continuous oral administration in normal rats. Leatham and Drill² employed intramuscular injection in normal and in hypophysectomized rats and observed hypertension. Recently Oster³ 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 Grollman⁶ 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 Leatham, 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.

TABLE I.
Systolic Blood Pressures of Treated and Untreated Rats for 24 Days.

Group	Substance injected	No. of rats	Sex	Range of B.P. mm Hg.	Mean B.P. mm Hg.
I	Diethylstilbestrol "	5	♂	80-128	110.8
		5	♀	80-135	116.5
II	Cottonseed oil "	5	♂	82-119	104.2
		5	♀	72-128	107.3
III	Untreated "	5	♂	90-128	111.4
		5	♀	80-125	107.5

no treatment for the same period of time and served as controls.

Systolic blood pressures were determined daily for 3 to 6 days before injections and then daily throughout the injection period and the week following treatment. Subsequent readings were obtained at intervals of several days up to the 78th day to determine whether there might be delayed effects.

Results. The effect upon blood pressure

of I diethylstilbestrol in cottonseed oil, II cottonseed oil and III control conditions in both male and female rats is presented in Table I. It is evident that although there was some variation both in the range and in the average of the blood pressures, there was no definite hypertension in any group. The blood pressure of the individual rats of each group also did not show a definite hypertension. The greatest variation of blood

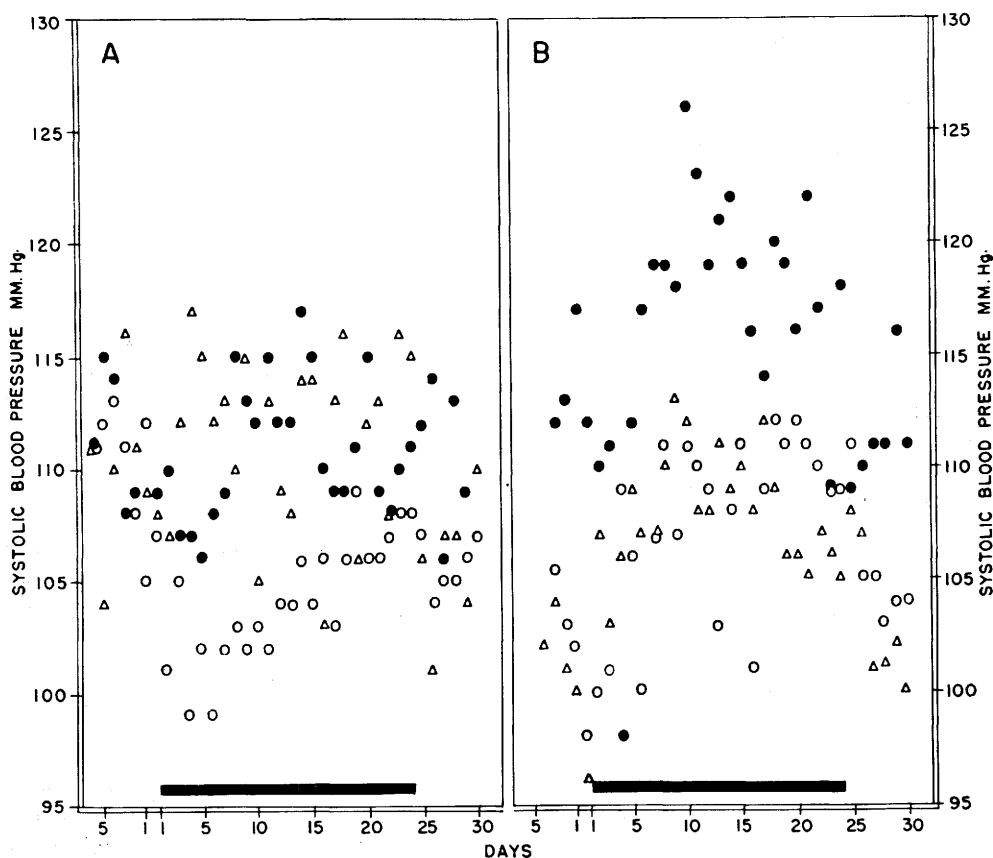


FIG. 1.

pressure of an individual male rat during diethylstilbestrol injection was 11 mm Hg. above the preinjection level and of a female rat 19 mm Hg. These variations are within the normal range for the rat.

There was, however, a slight difference between the blood pressures of the male and female groups. This is illustrated in Fig. 1, in which the blood pressures taken each day on the 5 animals in each group are averaged and plotted. The males are plotted under (A), and the females under (B). It is evident that the male rats receiving cottonseed oil had slightly lower blood pressures than the control rats or those receiving diethylstilbestrol. It is also evident that the blood pressures of the female rats receiving diethylstilbestrol are somewhat higher than the control or cottonseed oil-treated groups. The highest blood pressure readings were observed about the 10th day. Blood pressures obtained at intervals of several days and continued up to 54 days after the period

of injection revealed no further change.

Comment. These experiments have yielded results that are in accord with the report of Matthews, Emery and Weygandt⁴ that hypertensive levels of blood pressure do not occur in rats during continuous treatment with diethylstilbestrol. However, upward trends of blood pressure, not observed by these investigators, were observed in the present experiments. Hypertension of the degree reported by others¹⁻³ was not observed. Cottonseed oil when used alone slightly reduced the blood pressure of male rats, but did not affect the pressures of female rats. It, therefore, does not explain the slight elevation observed in the female rats treated by diethylstilbestrol in cottonseed oil.

Summary. Diethylstilbestrol did not induce hypertension in normal rats during 24 days of continuous intramuscular injection. Moderate but transient elevations of systolic blood pressure occurred in female but not in male rats.

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Some Cross-Circulation Experiments with Di- β -Chlorethyl Sulfide Poisoned Dogs.

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Death of animals caused by di- β -chloroethyl sulfide (mustard gas) has been considered by some workers¹ to be due to the liberation from poisoned tissues of a "secondary toxin." We have attempted to test this hypothesis by cross-circulation experiments with dogs.

When cross-circulation between a poisoned and an unpoisoned dog was begun after the original poison had disappeared from the blood of the first member, no symptoms of toxic action were evident in the second animal during a subsequent observation period

of 15 days. The dose used was about 30 times the LD₅₀ dose.

Experimental. Animals. Mongrel dogs of both sexes weighing 8-10 kg were used.

Cross-circulation. The animals were anesthetized with 30 mg/kg of nembutal intraperitoneally, and given additional small doses intravenously as required during the experiment. The left external jugular vein and left common carotid artery, of one dog, and the corresponding vessels on the right side of the other, were exposed. The 2 animals were then strapped together so that their exposed vessels could be conveniently joined. The distal end of the exposed portion of each vessel was ligated and the proximal end clamped with a bull-dog clamp. The ex-

* This work was done under contract with the Medical Division of the Chemical Warfare Service.

¹ Lewis, T. L., and Grant, R. T., *Heart*, 1924, 11, 209.