metrotropic activity as measured by uterine growth in immature mice*, however, these uterine-growth stimulating effects were not marked at doses below 100 μg where estrogen-inhibitory activities were quite apparent. Progesterone and 17-ethyl-19-nortestosterone appeared to be less potent than testosterone propionate in stimulating uterine growth, and all 3 have dose-response curves with shallow slopes similar to those of so-called impeded estrogens(4,7). It is of particular interest that a number of compounds which have dose-response curves with extremely shallow slopes will antagonize the action of estrone on the uterus. Preliminary studies on the effects of 17-ethyl-19-nortestosterone as an antagonist of estrone-induced vaginal cornification suggested that keratinization of epithelial elements proceeds in a relatively normal manner, but that leucocytes are never absent from the smears(8).

Summary. Testosterone propionate, progesterone and 17-ethyl-19-nortestosterone are all antagonists of estrone-induced uterine growth in intact immature mice. Progesterone has about 1.4 times and 17-ethyl-19-nortestosterone has about 70 times the potency of testosterone propionate. This high activity of 17-ethyl-19-nortestosterone did not appear to correlate with the androgenic, anabolic, progestational or metrotropic properties.

* Not published.

1. Dorfman, R. I., and Shipley, R. A., *Androgens,* etc., Wiley, New York, 1956.
2. Huggins, C., and Jensen, E. V., *J. Exp. Med.,* 1955, v102, 347.
3. Rubin, Betty L., Dorfman, Adaline S., Black, Lila, and Dorfman, R. I., *Endocrinology,* 1951, v49, 429.
4. Edgren, R. A., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 569.
5. Saunders, F. J., and Drill, V. A., *Endocrinology,* 1956, v58, 567.
6. Pincus, G., *et al., Science,* 1956, v124, 890.
7. Huggins, C., and Jensen, E. V., *J. Exp. Med.,* 1955, v102, 335.
8. Edgren, R. A., *Acta Endocrinologica,* in press.

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Strains of Small Race *Entamoeba histolytica* Maintained in Outdated Blood Bank Blood Medium.* (23005)

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Interest has been growing in culturing strains of small race *Entamoeba histolytica,* especially since new strains can be isolated and maintained easily(1). Although strains of large race have been cultured in media containing whole or defibrinated blood or red blood cells from various sources(2-10), strains of small race have not been grown in whole human blood. Recently, Tarshis(11) prepared a blood medium for growing *Mycobacterium tuberculosis.* It is the purpose of the present paper to describe 2 modifications of this medium which have been adapted for the maintenance of strains of *E. histolytica,* particularly those of the small race.

Materials and methods. 1. *Diphasic medium.* Outdated blood bank blood approximately 4 weeks old was used and contained ACD solution as follows: citric acid U.S.P., 0.50 g, sodium citrate U.S.P., 1.37 g, and dextrose U.S.P., 2.45 g/100 ml of transfusion solution.† The medium was made as follows: For basal medium, heat to dissolve 6 g of agar-agar in 276 ml of distilled water containing 4 ml of glycerol. Autoclave at 15 lb pressure for 15 minutes. Store in icebox or use immediately after by cooling to 45°C in

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† 480 ml of blood was collected in Baxter Transfuso-Vac bottles containing 120 ml of ACD solution.

water bath. Add immediately 4 ml of crystalline sodium penicillin G (160,000 units). Mix thoroughly; then add to mixture quickly 120 ml of 4-week-old blood which has been removed aseptically from the bottle by entering at "X" mark with appropriate syringes. Before the first entry into the bottle, briefly allow air to pass through sterile cotton-plugged needle plunged into "airway." Mix media thoroughly and dispense rapidly from sterile Kelly infusion bottle with bell-type filling attachment in 3.5 ml amounts into sterile screw-capped or cotton-plugged culture tubes. Make slants without butt and place them in incubator at 37°C for 24 hours to check for sterility. Overlay the slants with approximately 5 ml of buffered saline (pH 7.4) as previously described(1), by dispensing from a sterile Kelly infusion bottle supplied with bell-type filling attachment. Store media in icebox, and just prior to use bring it to room temperature and add a small amount of sterile Bacto-rice powder. 2. *Liquid medium.* The base for liquid medium was made as for the diphasic with omission of agar-agar. Blood, penicillin and 400 ml of sterile buffered saline were added and the medium dispensed in 6 ml amounts in sterile tubes. Rice powder was added, also. *Strains of E. histolytica tested.* The following strains of small race were carried on 48 hour schedule with 0.5 ml of sediment transferred: Griffith; Brown, a new strain isolated in July 1956 from stool specimen in charcoal slants; Conrad; and 110. K9, 200 and Komin large race strains were likewise maintained. The Komin strain was isolated from stool on charcoal slants in Sept. 1956. All strains were carried on egg slants(12) and in liquid charcoal cultures with 0.5 ml of sediment transferred every 48 hours. The egg slants served as controls. Isolation of new strains of large and small race from stools was not attempted on blood mediums.

Results. The strains of small race grown on egg slants and in liquid charcoal cultures multiplied well, but the majority of organisms were heavily vacuolated and rounded or crescent-shaped. Neither form showed much motility. The strains of large race, however, showed considerable motility in the same media.

In the diphasic blood medium all strains of small race showed exceptionally good growth with the greatest number of organisms in clumps of rice starch, and in starch which floated to top of the overlay. All strains of small race showed a decided increase in motility over the controls. The following are the subculture numbers for strains maintained in the diphasic blood medium: small races: Griffith-35; Brown-15; 110-17; Conrad-17; large races: K9-17; 200-17; Komin-24.

In the liquid blood medium, Griffith, Brown, and Komin, the only strains tested, showed sporadically good growth but these cultures were not maintained past the eighth subculture.

Generally, growth appeared greater in diphasic blood medium as compared to egg slant controls and liquid charcoal medium, but no comparative counts were made.

Discussion. The liquid medium was unsatisfactory for maintenance of strains of small race and large race as the red and white cells masked the trophozoites, especially small race trophozoites, many of which are not much larger than the red cells. In the diphasic medium, on the other hand, all strains tested showed excellent growth. Whereas in liquid charcoal medium and egg slants the small race trophozoite strains were non-motile or sluggishly motile, these same strains in diphasic blood medium showed increased degree of motility with many very actively motile forms. These actively motile forms often were difficult to observe unless they were in large clusters. It is interesting to note that amebae will grow so well in a medium to which old blood has been added.

Summary. 1. Two modified blood mediums, one diphasic, the other liquid, are described for growth and maintenance of *Entamoeba histolytica* of large and small races. The mediums are modifications of the one described and used by Tarshis(11) to grow *Mycobacterium tuberculosis.* 2. Strains of small race—Griffith; Brown, a new strain; Conrad; and 110—maintained in the diphasic medium exhibited increased motility as com-

pared to egg slant controls and increased growth over liquid charcoal cultures. The large race strains—200; K9; and Komin, a new strain—grew equally well in the diphasic blood medium. 3. The liquid blood medium was particularly unsatisfactory for maintenance of strains of small race, as the organisms were not easily discerned in the highly cellular sediment. 4. No attempts were made to culture stools or to isolate new strains of small or large race in either blood medium. 5. A 48-hour subculturing scheme was satisfactory for diphasic blood medium, liquid charcoal medium and egg slants; 0.5 ml of sediment was transferred.

1. McQuay, R. M., *Am. J. Clin. Path.*, 1956, v26, 1137.

2. Beltrán, E., and Ballesteros, E. Gutiérrez, *Rev. Inst. salub. y enferm. trop.*, 1950, v11, 107.

3. Drbohlav, J., *Ann. d. Parasitol.*, 1925, v3, 349.

4. Inoki, S., Nagai, A., Takeda, S., and Kitaura, T., *Handai-Ishi*, 1950, v2, 1.

5. Inoki, S., Takeda, S., and Nakabayasi, T., *Am. J. Clin. Path.*, 1953, v23, 197.

6. Kofoid, C. A., and Wagener, E. H., *Univ. Calif. Publ. Zool.*, 1925, v28, 127.

7. Kofoid, C. A., and McNeil, E., *Am. J. Hyg.*, 1932, v15, 315.

8. Phillips, B. P., *Science*, 1950, v11, 8.

9. Yamamoto, Y., *J. Oriental Med.*, 1937, v26, 937.

10. Yoshida, K., *J. Exp. Med.*, 1918, v28, 387.

11. Tarshis, M. S., *Am. J. Clin. Path.*, 1953, v23, 661.

12. Boeck, W. C., and Drbohlav, J., *Am. J. Hyg.*, 1925, v5, 371.

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Some Alterations in Serum Enzymes in Progressive Muscular Dystrophy.* (23006)

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Considerable attention has been given recently to the increase in activity of serum glutamic oxalacetic transaminase associated with tissue destruction. Examination of rat tissues by Cohen and Hekhuis(1) and Awapara and Sealer(2) showed this enzyme to occur in highest concentration in heart muscle. Skeletal muscle has about 3/4, and liver about 3/5 of heart muscle activity. Thus it is not unexpected that in diseases involving these tissues, such as myocardial infarction (3), acute liver injury(4) and in several muscle disorders(5) there is an elevation of serum transaminase activity. Schapira *et al.* (6) found that in progressive muscular dystrophy there is an increase in serum aldolase. There are several possible explanations for the increase in serum enzyme concentration; muscle destruction, the enzyme may have its origin in tissue other than muscle, and acceleration of protein turnover may result in an increase of enzyme release, producing a new balance between serum enzyme and the mechanism for enzyme disposal. By concurrent determination of serum aldolase and transaminase we have attempted to examine the first two of these possibilities. In addition alkaline and acid phosphatases were determined as representatives of a third group of enzymes.

Methods. Serum glutamic oxalacetic transaminase was assayed to modification of the method of Karmen(7) developed by Steinberg *et al.*(8). Enzyme activity was expressed as micromoles of oxalacetic acid formed/hour/ml of serum at 37°. Serum aldolase was determined by the Dounce *et al.* (9) modification of the method of Sibley and Lehninger(10). Following the suggestion of Beck(11) we extended chromogen reaction time from 30 to 60 minutes. The substrate employed was the magnesium salt of fructose

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