rapid separation of individual $C_{21}O_5$ compounds. The high degree of resolution obtained with these solvent systems is demonstrated in Fig. 2 by the wide separation of Kendall's E and 17-hydroxycorticosterone, both free and esterified, and also by the marked difference in movement of the acetates and propionates of each of these compounds.

Urine specimens, representing 72 or 96 hours' excretion, from 5 normal persons (4 males and 1 female), were continuously extracted at pH 1 with ether. The extracts were washed with dilute alkali and acid and then separately analyzed. By means of preliminary chromatograms in benzene:formamide, these neutral extracts were separated into three fractions containing, respectively, any C_2O_5, C_2O_4, or $C_{21}O_3$ corticoids that might have been excreted. Each $C_{21}O_5$ fraction was then rechromatographed beside pure compounds in toluene:propylene glycol. Two compounds whose movements were matched by Kendall's E and 17-hydroxycorticosterone, respectively, were found. Illustrations of these findings are given in Fig. 2 (A–D). These results were substantiated in all normals studied. The material eluted from appropriate areas of chromatograms not treated with the color reagent presented the ultraviolet absorption curve (maximum absorption at 240 mμ) typical of α,β-unsaturated 3-ketosteroids. The urinary compound whose mobility was the same as that of Kendall's Compound E also gave the blue color wit the iodine reagent. The other urinary compound, when eluted from the paper and dried, gave the yellow-green fluorescence with concentrated H_2SO_4 described for 17-hydroxycorticosterone (*3*). The eluted urinary compounds were also subjected to procedures for the preparation of the acetates and propionates of Kendall's Compound E and 17-hydroxycorticosterone and were then chromatographed beside the corresponding authentic esters. The alkaline silver solution was used as the color reagent. The derivatives of the urinary compounds moved at the same rates as the esters of the pure steroids (Fig. 2, E–L).

By rough matching of the size and intensity of the urinary spots with those of known quantities of pure Kendall's E and 17-hydroxycorticosterone, the normal 24-hr excretion of each was estimated to be between 20 and 40 μg. This was substantiated by measurement of the optical density at 240 mμ of the eluted compounds. In the normal persons studied, the excretion of Kendall's E appeared to be slightly greater than of 17-hydroxycorticosterone.

In the $C_{21}O_5$ fractions only the two compounds were found in 24-hr aliquots of the urine extracts. Thus any other steroid of this group, if present, was excreted in daily amounts under 10 μg, the minimum amount detectable with the alkaline silver reagent. No $C_{21}O_4$ or $C_{21}O_3$ corticoids could be found in aliquots of these extracts representing up to 72 hours' excretion.

In conclusion, the presence of Kendall's Compound E and 17-hydroxycorticosterone in the urine of normal persons is strongly indicated by: 1) the agreement in chromatographic behavior of the urinary compounds and the known steroids; 2) the similar movements of their esters; 3) the characteristic color reactions, and 4) the typical ultraviolet absorption curves. No other cortical hormone was found in appreciable amounts. The solvent systems described may be of value in the purification of synthetic cortical hormones by partition chromatography in starch or filter paper pulp columns.

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Cultivation of *Endamoeba histolytica* with *Trypanosoma cruzi*

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Endamoeba histolytica has been cultivated *in vitro* since 1925 (*1*), but always in the presence of bacteria. Cultivation of the ameba in the absence of bacteria is of utmost importance, since bacteria-free cultures are prerequisite for basic studies on the physiology and pathogenesis of this parasite, and on the serodiagnosis of amebiasis. During recent years several notable improvements in cultivation techniques have been accomplished, resulting in the acquisition of new information on growth requirements of the ameba. By the use of a microisolation apparatus, Rees and his associates were able to free cysts of *E. histolytica* from contaminants and thus to establish cultures of the ameba with various single species of bacteria (*2, 5*). Cultures with one species of bacterium designated as organism *t* have been used for preparation of antigens of high potency and specificity for the complement fixation test for amebiasis (*6, 12*). In addition, some information concerning the vitamin-cholesterol requirements of *E. histolytica*-organism *t* was obtained with the use of diphasic egg white medium and dialyzate medium (*6, 7, 13*). The work of Jacobs (*3*) demonstrated the usefulness of antibiotics in eliminating bacteria from ameba cultures and thus provided a basis for testing the effects of growth factors. Heat-treated *Escherichia coli* supported some growth of the ameba after the associated bacterium *Clostridium perfringens* had been eliminated with the use of penicillin and serial transfer. More recently, the work of Shaffer and Frye (*11*) demonstrated that good growth of the ameba may be obtained in wholly liquid medium with a species of streptobacillus inhibited with penicillin. The streptobacillus with an inhibiting dosage of penicillin was added at each serial transfer. The cultures of *E. histolytica* with live and heat-treated *Trypanosoma cruzi*, as described in the present report, were obtained by substituting the trypanosome for the bacterial associates used by previous investigators.

The growth of *E. histolytica* with *T. cruzi* was demonstrated in test tubes and in microtubes approximately

4 × 50 mm (*9, 10*). In both types of tubes a petrolatum seal was used to prevent access of atmospheric oxygen to the medium. The medium was composed of a thioglycollate preparation (Baltimore Biological Laboratory), horse serum, and the overlay of NIH diphasic blood-agar cultures (*4*) containing a rich suspension of *T. cruzi*. The F-22 strain of *E. histolytica* received through the courtesy of Drs. J. G. Shaffer and W. W. Frye, then of Vanderbilt University, was used in all experiments.

Test tubes containing the medium described were inoculated with amebas from cultures grown in the presence of penicillin-inhibited streptobacillus in a liquid thioglycollate-serum medium. Penicillin was used in the ameba-trypanosome cultures during the first four serial transfers to insure elimination of the streptobacillus and sterility tests were conducted at each transfer; these were negative. Control tubes without *T. cruzi* showed no growth of amebas. The ameba-trypanosome cultures have been maintained through 15 transplants at 48-hr intervals without any diminution in growth of the amebas. Rich cultures of amebas were produced in 48 hr and transplanted at the rate of six tubes from one. Active trypanosomes have been found to persist for as long as 10 days in these tubes, but clumping of the flagellates indicates that this medium is not entirely satisfactory for their growth.

The microcultures of *E. histolytica* were initiated from single trophozoites, washed free from components of the medium in sterile Locke's solution, and transferred by microisolation to microtubes containing the same medium as used in the test tubes, with the trypanosomes. As many as 200 amebas, the progeny of a single trophozoite, were counted by direct microscopic examination of the microtubes at 72 hr of incubation. Active amebas were seen for 10 days. Serial transfer of the microcultures was not carried out but the medium was transferred after 10 days to thioglycollate and neopeptone-broth media for tests of bacterial sterility. No growth of bacteria occurred in these tests.

Growth of *E. histolytica* with heat-treated *T. cruzi* has been demonstrated only in microcultures. The trypanosomes in the overlay of NIH blood-agar cultures were exposed to temperatures of 46° C for 1 hr 15 min in a water bath before addition of other constituents of the medium. Each microtube was inoculated with a single ameba trophozoite. In 20 microcultures thus far established, the ameba reproduced as rapidly as with non-heated *T. cruzi*. Transfer of the entire contents of the microtubes to NIH blood-agar medium gave no growth of trypanosomes.

The principal advantage of *E. histolytica-T. cruzi* cultures over ameba-bacteria cultures lies in the fact that *T. cruzi* may be inactivated at temperatures only a little higher than 37° C and hence without as much deleterious effect on proteins and enzyme systems as occurs in the heat-killing of bacteria. The cultures may therefore serve as a basis for the identification of growth factors required by *E. histolytica*.

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A Micromethod for Fat Analysis Based on Formation of Monolayer Films

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Lord Rayleigh (*5*) found in 1890 that lipids spread on water form films of wide extent, and Langmuir (*4*) found the water-insoluble fatty acid able to spread in monomolecular layers, with each molecule occupying an area of 20.5 square angstrom units, independent of the number of carbon atoms in the molecule.

Adam (*1*) showed that the spread of the film is little affected by temperatures from 20° to 30° C, if a film tension of 20 dynes per cm² is maintained.

The sterols, glycerides, phosphatids, cerebrosides, and hydrocarbons likewise spread, and the molecules occupy from 20 to 120 sq A. Mixed fats spread to about the same extent. Under controlled conditions, the area of this spread is a sensitive measure of the amount of fat. Gorter and Grendel (*3*) used this criterion to estimate the lipids of red cells.

It is relatively simple to estimate fatty acids, with a sensitivity and accuracy beyond other methods, by this spreading technique. A circular glass dish, 7½ in. in diam and ¾ in. deep, is supported on a small leveling table placed in an enamel tray, 12 × 14 × 1¾ in. The glass dish is coated with hard paraffin and filled with 0.3% H_2SO_4 (pH 1-2) until the level is above the edge of the dish.

The surface of this solution is cleaned by passing a glass slide (10 × 1 × 1/16 in.) from one edge to the other of the glass dish several times, wiping it dry each time. Droplets of piston oil are placed on the clean surface. They spread over the surface with a play of colors. Oil is added until the color is green. Such a film has been shown to have a uniform tension of 20 ± 2 dynes/cm². The piston oil is a good grade of lubricating oil heated to 300° C for 8 hr until it spreads as a monolayer (*2*).

The fat to be estimated is extracted from the original source by any good procedure that presents all the lipids in a clear petroleum ether solution in a volumetric flask of from 0.5 to 25 ml. From this volumetric flask a definite aliquot is placed by means of a capillary pipette