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

in the center of the piston oil film. As the solvent evaporates, the liberated fat spreads as a film against the pressure of the heavy oil film until equilibrium is reached.

A pane of glass is placed on the enameled tray, clearing the water surface by approximately ¼ in. A timer is started as soon as the fat solution is added. The film is allowed to age for 3 min. At the end of this time, by means of a wax pencil, the boundary of the film between the fat and piston oil is traced on the glass surface. A light placed directly above the dish aids in making this boundary distinct. The outline on the glass plate is readily traced on coordinate paper and the area is obtained by counting the squares; or if plain paper is used the outline is traced with a planimeter.

TABLE 1

Amount taken (in g)	Amount spread (in ml)	Spread (in sq in.)	Fat (equiv. in g)	Total fat found (in g)
0.0008	0.0052	3.6	0.0000054	0.00085
0.0018	0.0052	7.24	0.00001096	0.00173
0.0073	0.0052	31.28	0.00004692	0.00739

In the determination of small amounts of fat care must be taken in measuring the petroleum ether solutions. Small pipettes (containing from 0.005 to 0.1 ml) are used, that have narrow constrictions at the tip and at the calibrating mark to slow the flow of the ether. These are calibrated with mercury.

TABLE 2

Molecular weight	Area molecule (sq A)	Theoretical (1 sq in. = μg)	Found
Palmitic acid 256	20.5	1.33	1.35
Stearic acid 284	20.5	1.50	1.46
Cholesterol 390	41.0	1.02	1.05

In use, the pipettes are filled above the mark. The tip is wiped on filter paper and then held against the paper until the solution is brought to the mark. The solution is delivered on the surface with the last droplet forced out under the surface. The pipettes are rinsed with ethyl ether and dried with warm dry air in order to remove traces of moisture that render the measurement inaccurate. Repeated spreading of the same amount of lipid checks within 2%. This covers the pipetting of sample, the tracing, and the planimeter readings. Twenty-one such spreadings at 28° C gave a variation in areas of 1.6 ± .14% with a standard deviation of 0.96.

The sensitivity of the method is shown by Table 1, showing the analysis of skin fat, which was dissolved in 0.82 ml of petroleum ether. Aliquots were drawn with a pipette delivering 0.0052 ml. The factor was 0.0000015 g/sq in.

The important constituents of skin fat are fatty acids and sterols. These should agree with the theoretical spread. Using 20.5 sq A per molecule for fatty acids, and 41 sq A for cholesterol, the agreement is as shown in Table 2.

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A Preliminary Report on the Role of the Pineal Organ in the Control of Pigment Cells and Light Reactions in Recent Teleost Fishes[1]

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Although there has been a considerable amount of speculation on the possible functioning of both the pineal and the parietal organ in various fossil vertebrates, very little experimental work has been done on recent forms. Moreover, the precise function of the pineal organ in recent teleosts has never been clearly elucidated, although the structure is evidently present in all. A recent survey of the knowledge of the organ has been given by Gladstone and Wakeley (*5*). In fishes, all that is known concerning the relation of the pineal organ to pigment control is based on the work of von Frisch (*4*), which was confirmed by Scharrer (*7*). They demonstrated that the granules in melanophores of *Phoxinus* would disperse if the pineal region was stimulated by light. Since von Frisch was able to demonstrate that light sensitivity was not completely removed by extirpation of the pineal, and since he considered the lining of the ventricle of the diencephalon to have sensory cells, the exposed portions of the brain that react to light are here referred to as the pineal area. Parker (*6*) considered this fish exceptional. Scharrer demonstrated also that the pineal had an influence on phototaxis as well as on pigmentation, as did Young (*8*) for the very different, nonteleost, *Lampetra*. Breder and Rasquin (*3*) demonstrated an influence of the pineal on phototaxis for the blind cave fish *Anoptichthys*, which is responsible for the sign of the reaction.

Since the last work was published it has been possible to demonstrate that in a variety of eyed fishes dwelling in light, the pineal area has a distinct influence on both the behavior of the melanophores and the reactions of the fishes to light and darkness. It has been found that the fishes studied divide into three groups: those species in which the tissues, including the skull, overlying the pineal are sufficiently transparent or translucent to permit light to enter the cranial cavity freely over this region, as illustrated in Fig. 1; those in which the tissues

[1] This study will be reported in full detail at a later time. Most of the work was carried on at the Lerner Marine Laboratory at Bimini, and was in part supported by a grant from the American Cancer Society.