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Lipid Composition and Metabolism of *Trichomonas foetus*. (28272)

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Little is known of the metabolism of *Trichomonas foetus*, especially in the lipid field(1). The present paper investigates its lipid composition and metabolism.

Material and methods. *Trichomonas foetus* (TF) strains BR and 3P were used in this study. They were grown in modified Diamond's medium(2) containing proteose peptone, yeast extract (Difco) and 10% inactive horse serum. Agar was omitted. The parasites were propagated in 4 oz screw cap bottles containing 40 ml of medium. Incubation proceeded at 37°C for about 40 hours. At the end of the experiment, cells were harvested by centrifugation, and washed several times.

Extraction of the samples, column chromatography and radioactivity determination have been described(3). Lipid components of the various chromatographic fractions were detected by the Liberman-Burchard method (LB)(4) for sterols, and by methods used by Carlson *et al.*(5) for glycerol and by Knight *et al.*(6) for phosphorus. Phospholipids were calculated by multiplying the amount of phosphorus by 25.

Sterols were identified by co-chromatography using thin layer chromatographic method (TLC)(7). Chromatograms were developed with hexane - ethyl ether - acetic acid (50:50:2). Color was detected by spraying with $SbCl_3$ in ethanol. In some experiments sodium acetate-1-C^{14} specific activity 1 millicurie 12.6 mg (Volk Radiochemical Co.) was added to furnish 0.1 mc/100 ml medium. Experiments were repeated at least 3 times. The average results are presented.

TABLE I. Lipid Composition of *T. foetus*.

Strains	Sterol esters	Sterols	Glycerol	Phospholipids
		(μg per mg protein)		
BR	2.8	38.6	.37	77.5
3P	2.4	37.5	.36	77.5

Results and discussion. As seen from Table I, there was no appreciable difference in lipid composition between the 2 strains examined. Most of the sterols present were in a free form. The LB method distinguishes sterols on the rate of color development. The rate of color produced was that of a "slow acting" sterol, and was calculated as cholesterol. The amount of sterol present was about one hundred-fold more than glycerol, and about half the amount of phospholipids.

To elucidate further the nature of the present sterol, eluate from silicic acid column containing the free sterol was rechromatographed using the TLC method. After spraying with $SbCl_3$, a spot appeared on the chromatoplate. The color of the spot, its relative R_f value, and the rate of color developed with LB reagent, were similar to, or identical with, cholesterol. Lipid composition of the medium was also examined. The findings, mg per 100 ml medium, were: cholesterol esters 11.00, cholesterol 5.38, ergosterol 0.06, glycerol 0.37, phospholipids 11.00.

TABLE II. Incorporation of Acetate-1-C^{14} into Various Lipid Fractions of *T. foetus*.

Strains	Sterol esters	Sterols	Glycerides fatty acids	Phospho-lipids
		(% of C^{14} incorporation)		
BR	3.7	Traces	14.3	83.3
3P	3.3	"	17.1	79.6

The incorporation of radioactive acetate into various lipid factions is seen in Table II. As previously, there was no appreciable difference in incorporation between the 2 strains examined. Most of the radioactivity was incorporated into the phospholipids. Practically none was present in free sterols, and very little in sterol esters.

As appears from the results, the relative amount of cholesterol-like material is present in considerable amount in trichomonas, while no synthesis was demonstrated. As the medium contains an ample amount of cholesterol, it may serve as a source for this substance. This is in accord with the findings that trichomonads require sterol for their growth(8). It is of interest to note that while TF contain only "slow acting" sterol, several examined species of trypanosomidae such as *Leishmania tropica* and *Trypanosoma cruzi* contain considerable amounts of "fast acting" sterols(9).

The patterns of C^{14} distribution in lipids of TF incubated in the presence of radiocarbon labelled acetate was similar to that found in acetate injected chicks(3) and acetate fed rats(10).

Summary. Lipid content and metabolism of 2 strains of *Trichomonas foetus* were examined. The parasites contained cholesterol-like material in amounts of about one hundred-fold as much as glycerol and about half that of phospholipids. No synthesis of the sterol was detected.

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Binding of Fluorescent Insulin to Intracellular Antibodies in Guinea Pigs Immunized with Insulin.* (28273)

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The cellular aspects of antibody formation have been extensively studied(1-5) by means of the fluorescent antibody technique of Coons *et al.*(1), who found the indirect technique more sensitive than the direct method which employs fluorescent antigen. Nevertheless, demonstration of specific antibody in tissues by means of fluorochrome-labeled antigens under certain circumstances appears to offer a simple, direct approach to the study of antibody localization(6-9). The present report describes the preparation of fluorescein isothiocyanate conjugated insulin and the spe-

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