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Effect of Triparanol on Synthesis of Fatty Acids by *Tetrahymena pyriformis*.* (29300)

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Triparanol, an inhibitor of cholesterol synthesis in animals, also is effective as a growth inhibitor of protozoa(1). The triparanol-induced delay of synchronized first division(2) and growth inhibition in *Tetrahymena*(3) and *Ochromonas danica*(4,5) could be reversed by oleic or palmitic acids, among other compounds, suggesting interference with metabolism of long-chain fatty acids.

It was of interest to investigate the effect of triparanol on the synthesis of specific fatty acids, since Shorb(6,7) had demonstrated the synthesis of odd and even-numbered, as well as branched-chain fatty acids by *Tetrahymena pyriformis* in response to acetate, propionate, isobutyrate and α-methyl-*n*-butyrate as medium supplements.

Experimental. Strains H, S and W of *T. pyriformis* were grown in the dark at 24-26°C in 125 or 250 ml volumes in 500 or 1000 ml flasks (except as noted in footnote, Table I) on the synthetic medium D of Kidder and Dewey(8). The medium was modified by dilution to one-quarter strength; acetate, Tween 80 and glucose were omitted and vitamin B_{12}, biotin and thioctic acid were added (10, 3 and 10 μg/l, respectively). Growth supplements and inoculum treatment were as indicated in each experiment. Triparanol was used as an alcoholic emulsion, keeping alcohol to a minimum. Triparanol citrate was used as a water solution. The inhibitors were added only to the larger volumes of medium (250 ml) so that approximately equal numbers of organisms could be harvested around the mid-point of logarithmic growth. Because growth varied with the supplement added, the exact time of incubation is given in the footnotes of each table. Cells of *T. pyriformis* incubated with triparanol were more fragile than the control cells, making harvesting of the organisms more difficult.

Total lipids were extracted from centrifuged, frozen and thawed cells with chloroform-methanol (2:1, v/v) and methylated with BF_3 methylating reagent.‡ Fatty acid methyl esters were separated from other lipid components on a silicic acid column(9) with a benzene-hexane solvent (1:1, v/v). The methyl esters were further separated into saturated and unsaturated fatty acids by silicic acid chromatography of mercuric acetate adducts(10). Gas-liquid chromatograms of methyl esters were made on a 6 foot glass "U" column of 16% ethylene glycol succinate

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TABLE I. Effect of Incubation Period and Oleic Acid on Growth Inhibition by Triparanol.

Triparanol (μg/ml)	Oleic acid (μg/ml)	*T. pyriformis* strain W, Days of incubation 3	W 9	W 13	H 3	H 9	H 13
0	0	3+*	4+	4+	2+	3+	4+
1	0	±	3+	4+	±	3+	4+
3	0	0	±	+†	0	0	0
3	20	+	3+	4+	0	0	0

* ± to 4+ = barely visible to complete growth; 0 = no growth in 5 ml of basal medium in 20 mm diameter test tubes. The inoculum cultures, on unsupplemented basal medium, were grown to approximately ½ maximum growth and inoculated at 1 ml/100 ml of basal medium.
†Inhibited cells tend to break up.

on 80-100 mesh Chromosorb W‡ at 186°C, 15 psi inlet pressure of argon, in a Barber Colman instrument fitted with a radium detector. Tentative identification was made by comparison with pure fatty acids.

Results. Table I shows the effect of triparanol on strains W and H with and without oleic acid supplement. Both strains were inhibited with 1 μg/ml of triparanol up to 9 days of incubation; however, recovery from this inhibition occurred by 13 days. With 3 μg of triparanol and 20 μg/ml of oleic acid, only strain W recovered from the growth inhibition, whereas both strains were inhibited at 13 days without oleic acid.

Table II shows the effect of triparanol citrate and α-methyl-*n*-butyrate (AMB) on the amount of total lipids synthesized by 3 strains of *Tetrahymena*. Lipid synthesis by strain W was not affected by 0.6 μg/ml of triparanol citrate at 6 days of incubation. The inclusion of AMB to encourage the synthesis of branched-chain fatty acids(6,7) produced a slight inhibition of lipid synthesis. However, when both the butyrate and triparanol citrate supplements were present, greater inhibition of synthesis was seen. If incubation was continued for 8 days, the toxic effect of these two compounds was overcome.

TABLE II. Effect of Triparanol Citrate and α-Methyl-*n*-butyrate on Total Lipid Synthesis.

Supplements: Triparanol citrate (μg/ml)	Supplements: α-Methyl-*n*-butyrate (mg/ml)	Total lipids synthesized (mg/l of culture) by *T. pyriformis* strain W	S	H
.0	.0	94	105	69*
.6	.0	96		47*
1.2	.0		63*	
.0	.4	88		
.6	.4	48		
.6	.4	73†		
.0	.5		103	45*
.6	.5			39*
1.2	.5		31*	

* 7 days' incubation.
† 8 days' incubation; all others incubated for 6 days. Inoculum cultures were grown for one subculture on basal medium with or without α-methyl-*n*-butyrate. Butyrate inoculum was used for media containing the butyrate supplement.

Strain S required 1.2 μg/ml of triparanol citrate to produce ½ maximum inhibition at 7 days of incubation (Table II). The butyrate supplement had essentially no effect on total lipid synthesis, however, in combination with triparanol citrate, greater inhibition was seen at 7 days than with triparanol alone. Both triparanol citrate and the butyrate supplements were equally inhibitory to lipid synthesis in strain H when used separately, and decreased lipid synthesis only slightly more when used together. Additional experiments showed that AMB had a toxic effect on all 3 strains at various levels, but optimum growth could be attained if incubation was prolonged.

Total lipid synthesis and distribution of saturated and unsaturated fatty acids as influenced by triparanol were studied with other supplements to the basal medium and a lower level (0.3 mg/ml) of AMB (Table III). Incubations were extended to 8 days to increase lipid yield. Sodium acetate increased the total lipid synthesized over that of the basal medium, whereas sodium propionate and AMB depressed lipid synthesis. When triparanol was present, total lipid synthesis

TABLE III. Total Lipids Synthesized and % of Saturated and Unsaturated Fatty Acid Methyl Esters in *T. pyriformis* W Grown* with and without Triparanol on Different Medium Supplements.

	Medium supplement							
	None		Sodium acetate (.5 mg/ml)		Sodium propionate (.5 mg/ml)		α-Methyl-*n*-butyrate (.3 mg/ml)	
	Triparanol (1 μg/ml)							
	—	+	—	+	—	+	—	+
Total lipids synthesized (mg/l of culture)	98.6	20.4	127.4	43.9	58.1	5.3	45.1	12.3
% saturated fatty acids†	26	29	30	28	25	32	25	41
% unsaturated " " †	74	71	70	72	75	68	75	59

* Inoculum culture as in footnote to Table I. Experimental cultures incubated for 8 days.
† Separated as mercuric acid adducts on a silicic acid column (see text).

was inhibited in all cultures, but most markedly with the propionate and butyrate supplements. Triparanol greatly increased the % of saturated fatty acids synthesized with the AMB supplement, while only slightly increasing the synthesis with sodium propionate. A corresponding decrease in the unsaturated fatty acids was also observed. Triparanol had little effect on the ratio of saturated to unsaturated fatty acids in the unsupplemented or acetate supplemented medium.

Fig. 1 further illustrates triparanol interference with the synthesis of specific fatty acids. The lower curves are tracings of gas-liquid chromatograms of equal doses of the fatty acid methyl esters from total lipid extracts of strain W supplemented with AMB, with and without triparanol. Triparanol reduced the amounts of the C18:1,§ C18:2 and C18:3 unsaturated fatty acids as well as the C16:0 and C18:0 saturated acids. However, there was a substantial increase in the C13, C15 and C17 branched-chain and C16.7 saturated acids.‖ These values, expressed as % of total fatty acids, are tabulated in Table IV. The peaks (Fig. 1) normally occupied by C14 and C16 mono-unsaturated acids were saturated, as shown by the upper curve in the Figure of the saturated fatty acid methyl esters.

§ C18 refers to number of carbon atoms; :1, number of unsaturated bonds.

‖ The branched-chain fatty acids were identified originally by comparison with chromatograms of pure branched (anteiso) fatty acids, kindly supplied by Dr. Marjory Horning, Nat. Heart Inst., N.I.H., Bethesda, Md. C16.7, unidentified.

In contrast, the only noticeable change in fatty acid composition produced by triparanol in the acetate supplemented culture was an increase in the percentage of oleic acid (17 to 21%, analytical data not shown). With the propionate supplemented culture, triparanol increased the percentage of C17:0 and C19:0 acids and decreased the % of unsaturated fatty acids.

Discussion. Holz *et al*(3) suggested that the main site of triparanol inhibition of growth of *T. pyriformis* type II, var. 1 was the synthesis of unsaturated fatty acids. Triparanol blockage of synchronous division of strain G1 was reversed by oleic acid and Holz *et al* suggested oleic acid synthesis may be one prerequisite for division. Our work supports these observations, *i.e.*, triparanol

TABLE IV. Change in % Composition of Fatty Acids in Total Lipids of *Tetrahymena pyriformis* W Grown with and without Triparanol in Basal Medium Supplemented with α-Methyl-*n*-butyrate.

Fatty acid	Basal	Triparanol
C18:1*	17.6†	9.5
C18:2	23.6	18.5
C18:3	20.0	14.0
C16:0	6.3	5.3
C18:0	3.8	2.8
C13:br	1.3	3.8
C15:br	2.0	8.5
C16:7 (unidentified) }	4.6	13.1
C17:br }		
Others‡	20.8	24.5

* C18 refers to number of carbon atoms; :1, number of unsaturated bonds.
† The % of each fatty acid in total lipid of each culture was calculated from gas-liquid chromatograms of the methyl esters of mercuric adducts of unsaturated and saturated fatty acids.
‡ Several fatty acids, in the basal *versus* triparanol comparisons, differed by less than 1%.

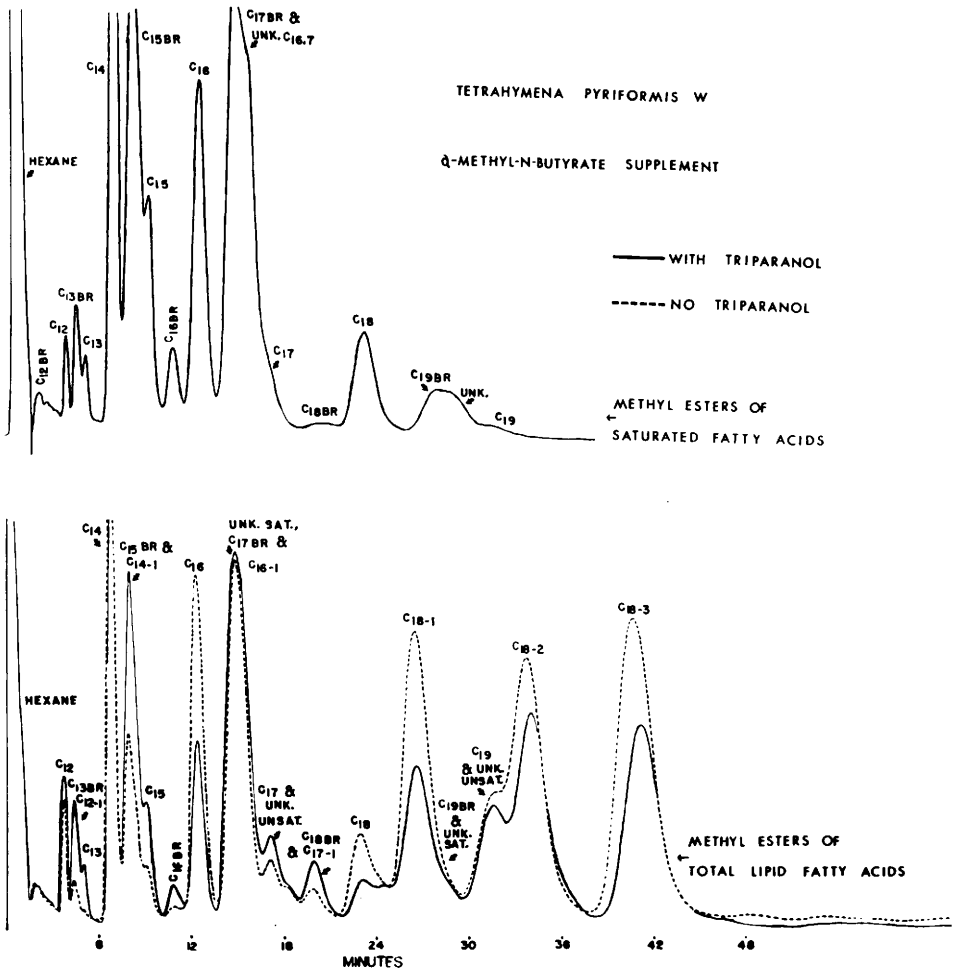

FIG. 1. Tracings of gas-liquid chromatograms of equal doses of total lipid methyl esters (lower curves) and saturated fatty acid methyl esters (upper curve) from *T. pyriformis* W grown with (———) and without (— — —) triparanol in α-methyl-*n*-butyrate-supplemented medium.

inhibited the synthesis of the total lipids in most experiments, inhibited the synthesis of unsaturated fatty acids, and increased the percentage of saturated fatty acids except in the acetate supplemented culture. The inclusion of certain precursors in fatty acid synthesis in the basal medium may influence the triparanol effect. While acetate alone increased total lipid synthesis, acetate plus triparanol inhibited total lipid synthesis although the % of oleic acid synthesized was increased by this combination. Strain differences in the response to triparanol and in the recovery from inhibition on continued incubation, may indicate the ability of some strains to adapt and to synthesize the unsaturated fatty acids at a faster rate than other strains. It is of interest that triparanol also stimulated fatty acid synthesis in rats (11) and in the serum phospholipids of pullets(12).

With the other supplements, propionate or α-methyl-*n*-butyrate, perhaps abnormal metabolites, the normal fatty acid conversion pattern was blocked by triparanol, and the proportion of odd-numbered and branched-chain saturated fatty acids was increased. Here triparanol may block the removal of the methyl groups from the branched-chain fatty acids and prevent dehydrogenation in the normal conversion to unsaturated fatty acids. Erwin and Bloch(13) established the

sequence of conversion of fatty acids in *Tetrahymena* species to be stearate → oleate → linoleate → γ-linolenate. They also observed that the branched-chain iso fatty acids disappear from *T. paravorax* cultures on continued incubation.

Triparanol also influenced the cell membrane, as evidenced by excessive fragility of cells in its presence. This perhaps is also mediated through interruption of the lipid synthesis or change in lipid composition of the cell wall. The interference of triparanol with the metabolism of fatty acids may explain some of the unfavorable side effects of triparanol treatment in humans. This is a field for further study.

The changes we have observed in the nonsaponifiable components of lipids of *Tetrahymena* inhibited with triparanol will be discussed later.

Summary. Triparanol suppressed the synthesis of total lipids in 3 strains of *Tetrahymena pyriformis*. Strain differences were noted when triparanol inhibition was reversed with oleic acid or by prolonged incubation. Supplements of short-chained carboxylic acids influenced the effect of triparanol on the total lipids, the percentage composition of saturated and unsaturated fatty acids and the specific fatty acids synthesized. There was an increase in the content of odd-numbered and branched-chain saturated fatty acids synthesized with triparanol plus propionate or α-methyl-*n*-butyrate supplements, and an increase in the percentage of oleic acid synthesized with triparanol plus acetate supplements. Triparanol may act by inhibiting the ability of *T. pyriformis* to demethylate and to dehydrogenate saturated fatty acids in formation of unsaturated fatty acids.

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Changes in Plasma Proteins After Portcaval Shunt in the Rat.* (29301)

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The electrophoretic distribution of serum proteins has been observed in a variety of clinical conditions. Severe malnutrition, wasting diseases and liver cirrhosis result in a decrease in albumin, attributed to impaired synthesis, and usually an elevation of the globulins. These changes occur in so many conditions that they are seldom useful for diagnostic purposes. The more specific hyperglobulinemia of multiple myeloma has been found to consist of a series of protein molecules in the γ group(1).

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