TABLE I. Composition of Endotoxins and Their Polysaccharides.*

No.	Type of products	N	P	Saccharide	Hexosamine	Total saccharide	Stearin equivalent
1	*E. coli* 0117:H27 LPSP	2.2	3.5	36.6	12.5	49.1	13.2
1A	Polysaccharide of same	2.3	2.4	52.3	24.2	76.5	0.0
2	*N. gonorrhoeae* 97-LPSP	2.1	3.4	22.0	20.3	42.3	23.2
2A	Polysaccharide of same	2.2	2.9	33.1	23.1	56.2	1.0
3	*S. abortus equi* LPSP	.8	2.1	47.0	7.7	54.7	11.2
3A	Polysaccharide of same	.8	2.0	60.0	4.7	64.7	0.0

* All values are given in per cent.

after hydroxylaminolysis. As expected, there was an increase in hexosamine and total saccharide content, after removal of the lipid moiety from the lipopolysaccharide molecule.

The ultracentrifuge patterns of all 3 polysaccharides reveal the presence of a major fast moving component and a minor slow moving component. The minor component is present in lowest concentration in the *N. gonorrhoeae* 97 sample. When graphically extrapolated to zero concentration of the specimens, the sedimentation constants (expressed in Svedberg units 1 S $= 10^{-13}$ cm/sec/units field) were as follows: for *N. gonorrhoeae* 97, $S_{20} = 8.3$; *E. coli* 0117:H27, $S_{20} = 9.2$ and *S. abortus equi*, $S_{20} = 9.4$. The rotor temperature was 20°C and solvent used was 0.1 M TRIS buffer, pH 8.0. Viscosity or diffusion measurements were not performed. Molecular weights were calculated from comparison of these sedimentation constants with those found for similar products and for which particle weights have been determined. Thus, the particle weight of the major component was found to be in the order of 200,000 and that of the minor component in the order of 10,000 or a small multiple thereof.

Summary. 1. There are marked differences between endotoxins prepared from 3 different species of gram negative bacteria. 2. The differences are retained after removal of the lipid component from the endotoxins by hydroxylaminolysis. Amino compounds are an integral part of both the endotoxin and polysaccharide molecules. 3. Molecular weights of the major components of the water soluble lipid-free materials are 200,000.

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Growth Inhibition of Microorganisms by Thyroid Hormones.* (26811)

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For unknown reasons thyroactive materials increase the vit. B_{12} (cyanocobalamin) requirement in rats, mice and chicks(1,2,3). This prompted the present investigation

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TABLE I. Effect of Thyroid Compounds on Growth Inhibition of *O. malhamensis*.

Thyroid compound (μg/ml)	B_{12} ($\mu\mu$g/ml)			
	No B_{12}	10	100	1000
None	.06	.6	2.1	3.0
L-Thyroxine				
.3	.06	.3	.92	2.8
1	.06	.12	.72	3.0
3,5-Diiodothyronine				
30	.06	.4	.9	2.8
100	.06	.14	.44	1.8
L-Triiodothyronine				
3	.06	.42	.96	3.0
10	.06	.14	.58	2.5
Tetraiodothyroacetic acid				
3	.06	.4	.8	1.3
6	.06	.22	.32	.32
3,5-Diiodo-L-tyrosine				
30	.06	.6	2.5	2.4
100	.06	.54	1.8	1.9

which reports some inhibitory effects of various thyroid hormones on growth of B_{12} and of non B_{12}-requiring microorganisms.

Materials and methods. The B_{12}-requirers used were (a) the mutant *Escherichia coli* 113-3, (b) *Lactobacillus leichmannii* ATCC 7830, (c) *Euglena gracilis* Z strain and (d) *Ochromonas malhamensis*. Methods for B_{12} assay with them have been described(4). The non B_{12}-requiring counterparts were: (a) *E. coli* ATCC 9637 (the parent strain of *E. coli* 113-3), (b) *L. casei* ATCC 7469, and (c) *Ochromonas danica*. Methods for growing these have been reported(5,6,7). Since all euglenids require B_{12}(8), non B_{12}-requiring controls for Euglena could not be included. Growth was recorded in optical density units by a Welch-Densichron equipped with a red-sensitive probe. The protozoa were grown at 28-32°C under light; bacteria at 37°C.

Results and discussion. Since only *O. danica* and *O. malhamensis* were affected by the thyroactive materials, detailed studies were confined to these 2 flagellates.

Ochromonas malhamensis. B_{12} overcame the growth inhibition induced by L-thyroxine, 3,5-diiodothyronine, L-triiodothyronine, tetraiodothyroacetic acid, and 3,5-diiodo-L-tyrosine (Table I); none of the sulfhydryl or other reducing compounds (Table II) or fatty acids (Table III) were active in this respect. The competitive inhibition index (ratio of inhibitor : metabolite) was approximately 300 for thyroxine, 3,000 for triiodothyronine and tetraiodothyroacetic acid, and 30,000 for 3,5-diiodothyronine. Iodinated L-tyrosine was included as a possible precursor of thyroxine; it was inert. Our findings suggest the possibility of using *O. malhamensis* as an indicator for thyrotoxic substances.

O. malhamensis has a B_{12} requirement like that of higher animals(9). When grown at the upper temperature limit its B_{12} requirement goes up steeply(10). This suggests, by analogy, that the B_{12} system in animals is strained under comparable metabolic stress (11). A B_{12} upset does exist in thyrotoxicosis(12).

Ochromonas danica. Because of the suggested interplay between B_{12} and glutathione in hyperthyroidism(13,14), some sulfhydryl compounds were included in this study. They did not counteract the inhibitory effects of the thyroid compounds on *O. malhamensis*, but *reduced* glutathione completely reversed inhibition of *O. danica* (Table II). Oxidized glutathione was not as effective as the reduced form; it did not overcome thyroxine inhibition, but was relatively effective against the other compounds. Thiouracil enhanced inhibition by all compounds. Vit. B_{12} did not reverse inhibition as it did for *O. malhamensis*. Thioglycolic acid and homocysteine overcame only diiodothyronine inhibition and cysteine overcame only the thyroxine and diiodothyronine inhibition.

Non-sulfhydryl reducing agents were also included in this study (Table II); none were as effective as *reduced* glutathione in overcoming inhibition. Ascorbic acid overcame diiodothyronine and tetraiodothyroacetic acid toxicity; it was slightly effective against thyroxine. Sodium metabisulfite was only effective against thyroxine inhibition.

Recent work on the protective action of fats on thyrotoxic rats showed that unsaturated vegetable fats promoted growth(15), an effect directly related to the linoleic acid content. Except for some reversal of triiodothyronine toxicity, linoleic, oleic, and stearic acids did not overcome the growth inhibition

TABLE II. Effect of Thyroid Hormones on Growth of *O. danica*.*

Compound (mg/ml)	Control	L-Thyroxine		3,5-Diiodothyronine		3,5,3'-Triiodothyronine		Tetraiodothyroacetic acid		3,5-Diiodo-L-tyrosine	
		30	60	100	300	10	30	10	30	100	300
No compound	1.72	1.22	.5	1.24	.88	1.56	.24	1.52	.14	1.44	.76
B_{12} (.001)	1.76	1.16	.6	1.44	.6	1.6	.36	1.68	.22	1.56	.72
Glutathione, reduced (.5)	1.8	1.88	1.92	1.82	1.78	1.88	1.84	1.72	1.72	1.7	1.58
Glutathione, oxidized (.5)	1.78	1.76	.48	1.84	1.76	1.8	1.2	1.8	.94	1.74	1.16
Thiomalic acid (.5)	1.7	1.6	1.82	1.72	1.8	1.78	1.34	1.76	.92	1.64	.68
Thiouracil (.5)	1.56	.08	.08	.1	.12	.62	.06	.08	.06	.6	.14
Thioglycollic acid (.2)	1.56	1.48	.68	1.72	1.8	1.62	.04	1.56	.32	1.52	.88
L-cysteine (.1)	1.76	1.64	1.46	1.64	1.44	1.72	.4	1.68	.34	1.42	.78
DL-Homocysteine thiolactone (.1)	1.8	1.56	.42	1.78	1.64	1.8	.42	1.76	.26	1.7	.66
Ascorbic acid (.5)	1.66	1.48	.92	1.66	1.72	1.76	.74	1.66	1.22	1.52	.34
Sodium metabisulfite (.05)	1.74	1.6	1.48	.14	.12	1.8	.3	1.0	.38	1.62	.24

* The thyroid hormones were added in concentrations indicated below for each compound in μg/ml.

of the thyroid compounds for *O. danica* (Table III); they were ineffective against inhibition of *O. malhamensis*. Polyoxyethylene sorbitan monolaurate (Tween 20), monopalmitate (Tween 40), and monooleate (Tween 80) were slightly effective; the trioleate ester (Tween 85) was completely effective in overcoming inhibition.

Animal studies have suggested that glutathione counteracts thyroxine inhibition by stabilizing endogenous vit. B_{12}(16). Lyophilized *O. danica* cells were assayed for B_{12}, but no measurable B_{12} activity was detected (the assay is sensitive to 10 $\mu\mu$g/mg of dried cells).

Mitochondrial derangement is corrected by B_{12}(16); it is postulated that B_{12} protects mitochondrial integrity by maintaining the sulfhydryl level. The absence of measurable B_{12} in *O. danica* may mean that the organism is more dependent on exogenous or endo-

TABLE III. Effect of Fatty Acids on *O. danica* Inhibition by Thyroid Hormones.

Thyroactive compounds (μg/ml)		Compounds (μg/ml)			
		No compound	Oleic, linoleic, linolenic, stearic acids (10)*	Tween 20, Tween 40, Tween 80* (50)	Tween 85 (50)
None		1.78	1.74	1.54	1.5
L-Thyroxine	40	.54	.64	1.14	1.66
	80	.22	.32	.84	1.68
3,5-Diiodothyronine	150	.54	.58	1.16	1.72
	300	.38	.22	.54	1.18
3,5,3'-Triiodothyronine	15	.84	1.2	1.26	1.76
	30	.14	.28	.5	1.58
Tetraiodothyroacetic acid	15	1.2	1.42	1.28	1.7
	30	.36	.4	.52	1.44
3,5-Diiodo-L-tyrosine	150	1.24	1.4	1.28	1.42
	300	.6	.66	.76	1.02

* Data given were obtained with the first compound of each group. Results with other compounds were similar.

genous sulfhydryl for mitochondrial protection.

O. danica resembles thyrotoxic animals in responding to fatty acids(15). Although linoleic acid was not active. Tween 85 permitted growth. The activity of Tween 85, contrasted with the inactivity of oleate and linoleate, raises the question of how much of the activity of Tween 85 is attributable to compounds other than the pure fatty acids used here. Since glutathione affects redox enzymes, the action of Tween 85 and glutathione may relate to their ability to maintain a redox potential favorable for mitochondrial function. Whether this involves the redox behavior of B_{12} itself remains to be seen. In Tetrahymena, a similar reversal of alkoxy-2,6-diaminopyrimidine inhibition has been shown for soy bean lecithin(17); the active components of the lecithin were not identified. The authors suggest that the lack of specificity of the lipid effect may mean that the inhibitors studies act as uncoupling agents. Such a possibility would apply with special force to thyroactive compounds(18).

Summary. The growth inhibition induced by thyroactive compounds on vit. B_{12} and non B_{12}-requiring microorganisms was studied. Thyroactive compounds were inactive for the B_{12}-requiring mutant *Escherichia coli* 113-3, *Lactobacillus leichmannii,* and *Euglena gracilis,* and for their non B_{12}-requiring counterparts. Only *Ochromonas malhamensis,* a B_{12}-requirer, and *Ochromonas danica,* a non B_{12}-requirer, were inhibited. Vit. B_{12} overcame the antagonistic action of the thyroid hormones for *O. malhamensis,* but not for *O. danica* where reduced glutathione and Tween 85 overcame the growth inhibition.

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Evidence for Immunization of F_1 Hybrid Mice Against Parental Transplantation Antigens. (26812)

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Generally implicit in transplantation experiments with mouse tissues has been the assumption that grafts of parental origin are nonantigenic to F_1 hybrid recipients(1-3).

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