TABLE I. Comparative Effects of Lyxoflavin and Liver Residue on Gain in Body Weight, Survival Rate and Organ Weights of Immature Hyperthyroid Rats (20 Rats on the Basal Ration; 10 Rats on Each of the Other Diets).

Supplements fed with basal ration	Initial body wt, g	Avg gain in body wt, g, on: 28th day	60th day	Surviving, %	Ovarian wt, mg	Adrenal wt, mg	Adrenal wt, mg/100 g body wt	Ventricular wt, mg	Ventricular wt, mg/100 g body wt
None	46.4	52.3 (12)	67.5 (8)	40	21.4	47.1	41.7	850	752
Lyxoflavin, 30 mg/kg of diet	46.1	56.8 (7)	75.7 (5)	50	21	51.2	41.8	982	801
Lyxoflavin, 100 mg/kg of diet	46.6	53.4 (7)	81.5 (2)	20	28.6	64.8	48.3	980	731
Liver residue	44.8	101.6 (10)	159.4 (8)	80	54.6	59.6	26.6	958	467
Basal ration without thyroid	45.2	90.2 (10)	152 (10)	100	47.3	44.5	22.9	658	338

Values in parentheses indicate number of animals which survived and on which averages are based.

conditions of the present experiment the antithyrotoxic activity of lyoflavin was not equivalent to that of liver residue. No data are available to indicate what factors are responsible for the diverse results obtained by Emerson and Folkers(5) and the present investigator. It is well established, however, that differences in the carbohydrate, protein and fat components of a diet exert a profound effect on the nutritional requirements of the hyperthyroid rat(6). The above investigations differed not only in the carbohydrate, protein and fat components of the diet but in the mineral and vitamin content as well. Differences also existed in the sex and strain of experimental animal employed. These differences may have contributed to the diverse results reported above.

Summary. Experiments were conducted on the comparative effects of lyxoflavin and liver residue on the gain in body weight, survival rate and ovarian weight of immature hyperthyroid rats fed a purified ration containing casein as the dietary protein and sucrose as the dietary carbohydrate. Liver residue prolonged survival and completely counteracted the retardation in body and gonadal weight observed in thyroid-fed rats on the purified basal ration. Lyxoflavin was without significant effect.

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Effect of Soya "Lecithin" on Number of Clostridia in Rat Feces. (19415)

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Recent studies with farm animals(1) and rats(2) have indicated that the putrefactive microorganisms of the intestinal flora may be undesirable. The dietary inhibition of putrefactive intestinal microorganisms has been accomplished by feeding lactose and dextrins

(3), low levels of protein(4), and by addition of antibiotics to the feed(1). Voet(5) reported that administration of lecithin to patients caused a decrease in odor of the feces. After confirming the observation in this laboratory by feeding soya "lecithin" to normal human subjects a study was made with rats to find out if the decrease in odor was due to changes in the intestinal flora. The present report describes an effect of soya phosphatides in reducing the number of clostridia in rat feces.

Experimental. Rats of the Sprague-Dawley strain maintained at constant temperature (25°C) in individual cages were fed *ad lib.* Twice a week, the diets were supplemented with 2 drops of oil concentrate containing vit. A (15,000 u), vit. D (2200 u), and α-tocopherol (10 mg) per g. Freshly dropped feces from individual rats were picked from blotting paper pads placed under the wire bottom of the cage. One g samples were added to 99 ml of sterile distilled water contained in dilution bottles and shaken mechanically for at least a half hour. From this 1-10^2 dilution further dilutions of 1-10^4 and 1-10^6 were made and plated on Wilson-Blair(6) medium. This medium has been used for the estimation of *C. perfringens* in water(6), soils(7), wounds(17), and feces(1). One ml of the dilution was pipetted into a Petri dish and thoroughly mixed with 15-20 ml of the warm dextrose-sulfite-ferric chloride agar. After the agar had hardened, a thin top layer of dextrose agar was poured. After 20 hours of anaerobic incubation at 35°C, the black colonies were counted. Brewer Jars containing 90% N_2 and 10% CO_2 were used for maintenance of anaerobiosis. The crude soya "lecithin"* containing 2/3 crude phosphatides and 1/3 soybean oil was homogenized into skim milk supplying an equal weight of solids and spray dried; hence the dried "lecithin" compound used in the preparation of diets contained 50% skim milk solids, 33% phosphatides and 17% soybean oil.

Results. In a preliminary experiment, the soya "lecithin" 50% skim milk 50% compound was fed at a 25% level in a Rockland Rat Diet.† After 6 and up to 24 days on the "lecithin" diet the average Wilson-Blair plate count (5 rats, 30 determinations) per g of feces was 8,000; the range was from negative plates at 1-100 dilution to a maximum of 38,000. Parallel determinations on the control diet averaged 12 million with a range from negative at 1-100 dilution to a maximum of 159 million. The feces of the "lecithin" fed group contained on the average only about 1-1000th of the number of clostridia present in the feces of the control group. However, the low counts present in the feces of some members of the control group obscured the significance of the data and so control diets were sought which would result in high count in all rats.

A diet of horsemeat was found to increase consistently the Wilson-Blair plate counts of the feces from 3-30 (avg 13) millions per g(4). Addition of 12.5% skim milk powder to the horsemeat increased the fecal counts to higher levels 30-90 (avg. 57) million per gram. Addition of 12.5% soya "lecithin" to the skim milk-horsemeat diet reduced the clostridia counts after one week of feeding and during a period of 5 weeks (40 determinations on 5 rats) to an average of 200,000 colonies per g, approximately 1-300th of the control values.

To confirm these results, to find out about the reversibility of the effects, and also to obtain information about the constituents of soybean lecithin responsible for the decrease in clostridia, the following experiment was conducted. Forty male weanling rats were fed the horsemeat-skim milk diet for 24 days. The 30 rats having the highest fecal clostridia counts as determined by 2 counts during the last 2 weeks of this period were divided into 6 groups, 5 rats per group. Diets having the composition listed in Table I were then fed. From the data of Scholfield *et al.*(8) the amounts of choline and inositol supplied by diet L were calculated and these amounts were added in diets Ch, I, S, and M_1. Diets containing these components of "lecithin" phosphatides were tested because of their possible influence on intestinal flora. Inositol has

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TABLE I. Composition of Diets Fed (g/kilo).

	C	L*	S	Ch	I	M_1
Horsemeat	875	750	833	872	872	827
Skim milk solids	125	125	125	125	125	125
Crude phosphatides	—	83	—	—	—	—
Soybean oil	—	42	42	—	—	42
Choline	—	—	—	2.75	—	2.75
Inositol	—	—	—	—	3.25	3.25

* Diet prepared by using 25% "lecithin" compound described in text.

TABLE II. Clostridia Count of Feces of Rats Fed Various Diets (Millions/g of Feces).

Dates: Diet* changes	Dates: Determinations	Group No. 1	2	3	4	5	6
8/27		C	C	C	C	C	C
	9/12	34	16	40	22	9	43
	20	26	44	20	38	51	17
	Avg	30	30	30	30	30	30
10/2		C	L	S	Ch	I	M_1
	10/9	93	95	95	182	130	60
	16	115	4.6	71	88	86	76
	23	22	1.2	1.7	23	60	37
	30	100	.006	11	14	114	13
	11/6	65	.13	74	44	126	44
	13	10	.004	31	15	72	2.7
	20	44	.016	13	19	57	11
11/22		C	C	M_1	M_1	M_1	L
	11/27	66	47	42	38	42	.3
	12/3	39	33	35	46	59	.014
	12	34	81	79	76	55	.91
12/12		C	L	L	L	L	L
	18	25	.27	.47	.23	.24	.01
	26	35	.006	.20	.004	.012	.83
12/27			C	C	C	C	C
	1/7		43	58	48	58	56

* Composition of diets listed in Table I.

been shown to change intestinal flora(12). The average fecal plate counts of the rats obtained as the result of feeding these diets are shown in Table II.

Inspection of data of Table II shows that only diet L containing soya phosphatides caused a significant reduction of the fecal clostridia counts. The average number of clostridia organisms per g in the feces of rats fed diet L (250,000) was about 1-200th of the average number present in the feces of the control group (47 million). In 90 determinations of the feces of "lecithin"-fed rats negative plates at 1-100 dilution occurred 22 times. The highest counts observed were 3 and 4 million, both occurring in one rat of Group 6. In the other 24 rats no values over 1 million were observed. The effect of dietary soya phosphatides in reducing the fecal clostridia count appeared as early as the 5th day of feeding, reached maximum after 2 weeks and was maintained during administration for as long as 30 days. The effect was reversible; removal of the phosphatides from the diet caused the fecal counts to rise to control values.

In a continuation of the study it was found that addition of ethanolamine either to the control diet (11 g/kg) or to diet M_1 (2.7 g/kg) resulted in no change of fecal clostridia counts from control values. Colonies picked at random from plates from both the control and "lecithin" fed groups when inoculated into milk resulted in stormy fermentation in 98 out of 135 trials. This result indicates the predominating species present was *C. perfringens*(9).

Attempts to demonstrate an *in vitro* effect of soybean "lecithin" phosphatides on clostridia cultures were unsuccessful as were attempts to demonstrate an inhibitory factor towards clostridia organisms in the feces of the rats fed soya "lecithin." No significant differences in the pH of the feces of the rats on the different diets was observed.

Studies were also made of the effect of the diets on the numbers of fecal coliform and proteus organisms. The number of fecal coliform organisms was estimated by EMB media plate counts(10). The average fecal EMB counts on the "lecithin" diet (0.8 million/g) were about ¼ of the average counts (3 millions/g) on the control diet. Estimations of the numbers of proteus in the feces were made with ricinoleic acid media(11). The average number of proteus per g in the feces of the control group and "lecithin" group was 25000 and 85000 respectively.

Discussion. The addition of penicillin and terramycin to pig and turkey diets results in increased growth accompanied by a great reduction of the number of clostridia organisms in the cecal contents and feces(1). Clostridia have been implicated as the cause of enterotoxemia in sheep(12), "6 day disease" of chicks(13), and gastrointestinal disease in humans(14). Clostridia are present in the feces of artificially fed infants in large numbers but are absent in the feces of breast fed children(15). Clostridia counts are higher in older adults(16). Administration of soybean "lecithin" with a consequent long lasting reduction of the numbers of intestinal clostridia may be a method for evaluating the effect of these organisms on growth and in disease.

Summary. The feces of rats fed a diet of horsemeat or horsemeat and dried skim milk, regularly contained a number of anaerobic micro-organisms which form black colonies on Wilson-Blair medium plates. These organisms were presumed to be mainly clostridia, especially *C. perfringens*. Addition of a soybean "lecithin" compound (soybean "lecithin" homogenized in skim milk and dried) supplying 8.3% phosphatides to a horsemeat-skim milk diet greatly reduced the clostridia count of the feces. Under the same conditions, the addition to the diet of soybean oil, choline, inositol or ethanolamine singly or in combination at the same levels supplied by the soybean "lecithin" compound resulted in no significant change in the number of these organisms. Therefore, the reduction in fecal clostridia count observed on addition of soybean lecithin to the diet was probably due to one or more of the soybean phosphatides.

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