compound was not prolonged and its excretion via gastro-intestinal tract was negligible, it is concluded that the compound is to all practical purposes completely metabolized in the body.

The low urinary excretion of tripelennamine is not unlike results we have found with other antihistamines, diphenhydramine (Benadryl), phenindamine (Thephorin) and pyranisamine (Neo-antergan), which are excreted in the urine only to a minor extent after oral administration(10,11).

Role of the liver in the metabolism of tripelennamine. Preliminary studies *in vitro* and *in vivo* indicate that the liver is important for metabolizing tripelennamine. In studies with tissue slices of various organs, the liver was found to be by far most active for altering tripelennamine. Less than 10% of tripelennamine was recovered after aerobic incubation of 20 μg of the compound for 2 hours at 38° with 200 mg of liver slices suspended in Krebs-Ringer solution. In 2 rats which were partially hepatectomized, severe convulsions were noted and one animal died after the administration of 40 mg/kg of tripelennamine intraperitoneally, whereas this did not occur when 2 laparotomized rats were given the same dose. Also, in our distribution studies with normal animals, convulsions were not noted after 50 mg/kg of tripelennamine.

Summary. Tripelennamine (Pyribenzamine) was estimated by the Brodie methyl orange technic which was modified to give a high degree of specificity. The compound is rapidly and completely absorbed by rats following oral administration. It rapidly leaves the blood and localizes in all tissues especially lungs. Brain, kidneys, spleen, liver and heart all may concentrate appreciable amounts, the level of tripelennamine present in each organ being somewhat dependent upon its mode of administration. High brain levels of tripelennamine were consistently noted with lethal doses of the compound. Prolonged storage of the compound does not occur and only small amounts are excreted via the urine and feces. It is concluded that the compound is almost completely metabolized in the body. The liver subsequently was found to be the most active organ for metabolizing tripelennamine.

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10. Leong Way, E., Overman, J. R., and Howie, D. L., *Fed. Proc.*, 1947, v6, 382.

11. Unpublished findings.

Effect of Feeding Polyoxyethylene Monostearates on Growth Rate and Gross Pathology of Weanling Hamsters.* (17703)

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Considerable interest has developed in studying the effects of ingesting emulsifying agents such as polyoxyethylene derivatives of fatty acids on the assimilation of various lipoidal materials(1). The present study was undertaken to determine the effect of feeding polyoxyethylene monostearate preparations on the growth rate, food efficiency and gross pathology of weanling hamsters.

Experimental. Weanling hamsters† (4-6 weeks of age) of mixed sexes were placed in individual cages in an air-conditioned room

* We are indebted to Mr. Donald Kraybill and Mr. Roy Ring for their assistance in the care of the animals.

1. Anon., *Nutrition Rev.*, 1949, v7, 205.

† Obtained from the Haskins Rabbitry, St. Louis, Mo.

TABLE I.
Composition of Basal Diet.

Constituent	%	Constituent	mg/100 g
Purified casein	24.0	Thiamine hydrochloride	0.6
Sucrose	62.4	Riboflavin	0.6
Celluflour*	3.0	Pyridoxine hydrochloride	0.6
Salts IV†	4.0	Ca pantothenate	4.0
Fortified cod liver oil‡	0.3	Nicotinic acid	2.0
1:20 Liver extract§	1.0	Choline chloride	100
Cystine	0.3	Inositol	100
Corn oil	5.0	Pteroylglutamic acid	0.2
		Biotin	0.01
		p-aminobenzoic acid	30
		2-methyl-1,4-naphthoquinone	3.0

* Chicago Dietetic Supply Co.
† Hegsted *et al., J. Biol Chem.*, 1941, v138, 459.
‡ 6000 I.U. of vitamin A and 850 I.U. vitamin D per g. Alpha tocopherol was added to supply 2.5 mg per 100 g diet.
§ Wilson Laboratories, Chicago.

maintained at 76°F. All animals were fed a purified diet for a preliminary period of 2 weeks. This diet was designed to be adequate on the basis of information available on the nutrition of the hamster(2,3) and the composition is indicated in Table I. At the end of this 2-week period the animals were randomized among the experimental groups on the basis of weight, gain in weight and sex. Thirty-three animals were selected for each group. The basic diet used during the experimental period of 10 weeks differed from the diet fed during the preliminary period in that the corn oil was reduced from 5% to 1% of the total diet. The appropriate test products were then added at the expense of the sucrose. Two polyoxyethylene monostearate preparations were tested (Sta-soft and Myrj 45) and the results were compared with those obtained with prime steam lard. The following dietary regimens were studied (*ad libitum* unless indicated otherwise): I—5% lard; II—5% Sta-soft; III—5% Myrj; IV—5% lard with the food intake restricted to the amount consumed by the average of Group II or III whichever was the least; V—15% lard; VI—15% Sta-soft; VII—15% Myrj; VIII—15% lard with the food intake restricted to that of Group VI or VII whichever was the least; and Group IX—7½% lard plus 7½% celluflour. The latter diet was included in order to evaluate the effect of feeding a material regarded as essentially inert nutritionally (celluflour) at a level to approximate the non-fatty acid moiety of Sta-soft and Myrj. The lard, Sta-soft and Myrj were kept at all times in a cold room (23°F) and small amounts were removed, melted and incorporated in the diets which were mixed once a week. These diets were then refrigerated at 36°F when not in use. All food remaining in the food cups was discarded at the end of each week and clean food cups provided. The rate of gain, food consumption, mortality, gross pathology of all animals that died during the experiment, and the gross pathology and organ weights of the animals sacrificed at the end of the experiment were determined.

Results and discussion. The results for the rates of gain obtained for the ten weeks on experiment are presented in Fig. 1 and 2. The results obtained for the food consumption, food efficiency and mortality are presented in Table II.

It can readily be seen that the ingestion of 5 or 15% Sta-soft or Myrj resulted in slower rates of gain as compared to the ingestion of lard, fed *ad libitum,* and this effect was quite apparent at the end of the second week on experiment. The growth data obtained for Group I (5% lard) and Group II (5% Sta-soft) were analyzed statistically. The F value obtained by an analysis of variance was 19.2 (P = less than 0.01). These groups

2. Hamilton, J. W., and Hogan, A. G., *J. Nutrition,* 1944, v27, 213.
3. Schweigert, B. S., *Vit. and Hormones,* 1948, v6, 55.

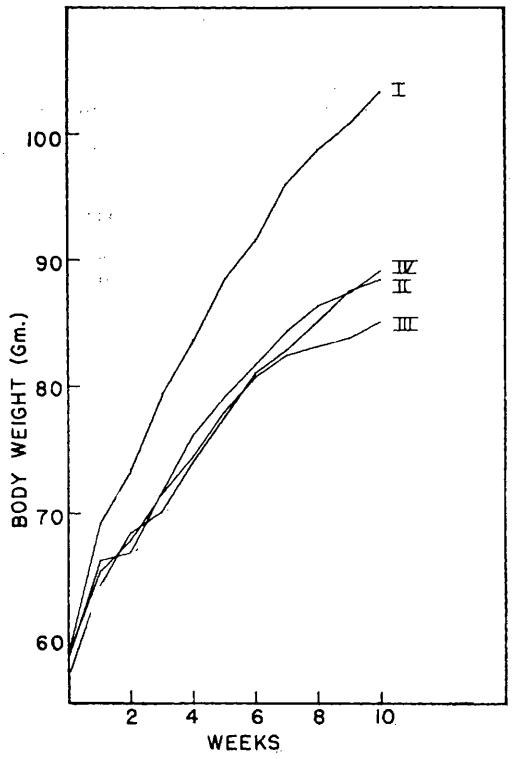

FIG. 1.
Growth curves for animals receiving 5% lard (Curve I), 5% Sta-soft (Curve II), 5% Myrj (Curve III), and 5% lard, food restricted (Curve IV).

were selected since the difference in the mean gains was the least (*i.e.*, 4.4 and 3.1 g per week respectively, as compared to 2.7 g for the animals fed 5% Myrj). The differences in the gains for the groups fed 15% of the test products were even greater. It was concluded, therefore, that the growth rate was significantly reduced and attributable to the ingestion of the Sta-soft and Myrj.

It should be pointed out that the food restriction experiments were not completely successful in that a few of the animals in Group IV did not eat the amount of food determined as the average consumption of Group II or III. Consequently, the group average for Group IV was reduced. It is of interest, however, that even though the food intake was the lowest, the gains were essentially the same as for Group II or III. The superior performance of Group IV as compared to Group II or III is apparent from the food efficiency data (Table I). A separate calculation was made for animals in Group IV that consumed all the ration which was equivalent to the food consumed by Group III, and the average gain per week was 3.5 g (g gained per gram food consumed x 100 = 9.3).

It will also be noted that Groups VI and VII consumed more food than Group V; consequently, the food intake of Group VIII could not be restricted to that for Group VI or VII. Thus Group VIII was essentially an *ad libitum* fed group as most of the animals did not eat the food allotted on the basis of food consumed by Group VI or VII. This is clear from the data on the rate of gain, food consumption and food efficiency, which are essentially the same for Groups V and VIII (15% lard). The group receiving 7½% lard and 7½% celluflour made excellent gains; in fact the best for any group. The food

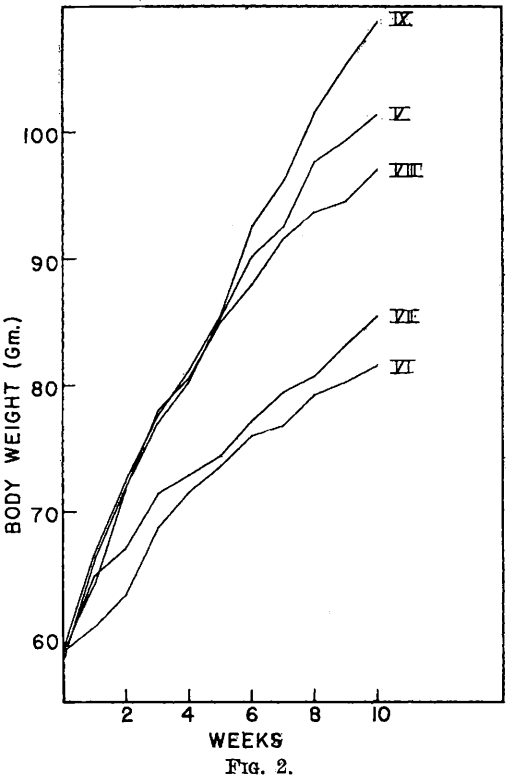

FIG. 2.
Growth curves for animals receiving 15% lard (Curve V), 15% Sta-soft (Curve VI), 15% Myrj (Curve VII), 15% lard, food restricted (Curve VIII), and 7½% lard plus 7½% Celluflour (Curve IX).

TABLE II.
Summary of Data on Rate of Gain, Food Consumption, Food Efficiency, and Mortality.

Group No.	Test product	Food consumption, g/day	Food efficiency, g gain/g food consumed ×100	Mortality
I	5% Lard	5.9	10.7	None
II	5% Sta-soft	5.7	7.3	4
III	5% Myrj	5.4	6.4	4
IV	5% Lard (food restricted)	5.2	8.3	None
V	15% Lard	5.0	11.4	6
VI	15% Sta-soft	5.5	4.9	10
VII	15% Myrj	5.5	6.0	5
VIII	15% Lard (food restricted)	5.0	10.6	2
IX	7½% Lard 7½% Celluflour	6.2	10.4	4

TABLE III.
Comparison of Organ Weights with Body Weights of Hamsters Fed Various Diets.

Test product	Wt of organ per 100 g of body weight			
	Liver, g	Kidney, g	Spleen, g	Testes, g
5% Lard	4.78	.43	.14	.75
5% Sta-soft	5.56	.53	.14	.60
5% Myrj	4.98	.52	.16	.33
5% Lard (food restricted)	5.01	.46	.15	.34
15% Lard	4.68	.41	.17	1.01
15% Sta-soft	6.03	.59	.16	.45
15% Myrj	5.45	.55	.15	.54
15% Lard (food restricted)	4.38	.43	.19	.56
7½% Lard + 7½% Celluflour	5.06	.41	.17	.61

consumption was also the highest and the food efficiency paralleled the data for Groups I, V and VIII, which received lard without additional celluflour.

Thus the animals in Groups VI and VII compensated at least in part for the apparent caloric inertness of the polyoxyethylene portion of Sta-soft and Myrj and of the additional celluflour in the case of Group IX by consuming more total food. This did not impair animals' performance when receiving celluflour (Group IX), but in spite of the fact that the animals consumed more total food when fed Myrj or Sta-soft, the rates of gain were markedly inferior to those for animals fed lard. It is apparent, therefore, that an inadequate food intake was not the explanation for the inferior performance of the animals fed Myrj or Sta-soft, and that the ingestion of the polyoxyethylene fraction of these products did not show the same results as feeding a substance regarded as essentially inert in caloric value (celluflour).

Although it is difficult to classify the gross pathology noted in a specific sense, it was consistently evident that profound changes in the gastrointestinal tract occurred in all animals fed Myrj or Sta-soft. These changes ranged from a mildly distended intestinal tract with the contents of the lower bowel fluid to a greatly distended intestinal tract with large amounts of gas and fluid present. The cecum was greatly enlarged and for many animals fed 15% Sta-soft or Myrj, the ceca were approximately 4 times the size of those from animals fed lard. Evidence for the presence of blood in the cecal contents was observed for 2 of the animals fed 15% Myrj, indicating hemorrhage. The liver from one animal fed 5% Sta-soft was necrotic and the kidneys from 2 animals fed Sta-soft and 2 animals fed Myrj were grossly abnormal with hemorrhagic areas apparent as well as severe crater-like depressions approximately 1 mm in diameter. Thus, it is clear that not only were differences in the growth rate and effi-

ciency of gain noted, but also differences in gross pathology when hamsters were fed Myrj or Sta-soft as compared to lard.

Evidence of diarrhea and inflammation at the base of the tail was apparent after the animals were fed 5 or 15% Myrj or Sta-soft for 7-10 days. Animals receiving lard were not affected. The mortality was highest in the groups fed Myrj or Sta-soft during the first 4 weeks of the experiment. After this time, the effects seemed to become less severe, the diarrhea or fluid fecal condition was present continuously but did not appear to affect the surviving animals as much as during the early phases.

It will be noted that some animals receiving 15% lard (*ad libitum* and restricted food intake groups) and 7½% lard and 7½% celluflour died during the experiment. The deaths followed no consistent pattern and were observed throughout the test. No good explanation is available for these findings. The rest of the animals showed no effects at the time of the deaths, and although a few showed some evidence of a diarrhea condition, it was of a different nature than observed for the groups fed Myrj or Sta-soft. Nevertheless, no animals receiving 5% lard died, while the corresponding losses in the groups fed 5% Myrj or Sta-soft were 4 and 4 respectively. Considering the groups as a whole, 12 out of a total of 165 hamsters receiving lard died or 7.3% from all causes, while 23 out of a total of 132 receiving 5 or 15% Myrj or Sta-soft failed to survive or 17.4% from all causes. Therefore, although it is quite evident that a higher mortality occurred when Myrj or Sta-soft was fed as compared to lard, the results cannot be considered unequivocal by themselves. They are indicative and strengthen the overall conclusions when considered with the other objective data obtained.

Sections of the small and large intestine, liver and kidney were taken from a representative animal in Groups I, II, III, V, VI and VII at 2, 4, 6, 8, and 10 weeks on experiment for histological examination. Sections were taken from all groups at 6 and 10 weeks. These studies will be presented at a later date.

At the conclusion of the experiment the weights of the liver, kidney, spleen and testes were taken. In these studies, the animals were sacrificed by decapitation, and the organs weighed immediately after dissection. Gross pathological findings were also noted. The cecal contents of two animals receiving 15% Myrj gave positive tests for blood (benzidine test). Representative tests conducted on animals in other groups were negative. The number of animals taken for measurements of organ weights was limited to 18, which included 9 males, since this was the number that survived in Group VI. It should be pointed out that these weights are for the entire liver and spleen, one kidney, and both testes. The data compared on a body weight basis are presented in Table III. No differences were noted in the weights of the livers, kidneys, and spleens for the males and females; consequently, the composite data are presented.

The weights of the liver on a body weight basis are higher for the groups fed 5% Sta-soft (Group II), 15% Sta-soft and 15% Myrj than for the corresponding groups fed lard. Slight increases in the kidney weights were apparent for the groups fed 5% Sta-soft or Myrj and consistent increases for the group fed 15% Myrj or Sta-soft. The data for the groups fed 5% lard were very similar to the data for the animals fed 15% lard. The data obtained for the testes indicate that the animals fed Myrj or Sta-soft had somewhat smaller testes. There was considerable variation in these weights and, further, the limited number of animals studied(9) and lack of agreement in the data for the different groups fed lard do not permit definite conclusions to be made.

It is recognized that there are limitations to evaluating these data only on the basis of weight per animal since the animals fed lard were larger and would be expected to have correspondingly larger organs. Conversely, by calculating the data per 100 g of body weight it is assumed that the ratio of body weight to organ weight is constant, an assumption which is not based on experimental evidence obtained with hamsters. This is quite valid for the animals fed lard, since

they approximated 100 grams but the animals fed Myrj or Sta-soft approximated 85 grams in weight. Therefore, these data are regarded as indicative, but not conclusive.

Summary. The ingestion of 5 or 15% of polyoxyethylene monostearate preparations (Myrj of Sta-soft) by weanling hamsters significantly reduced the rate of gain as compared to animals fed lard. This was evident after 2 weeks on experiment. There was also a decrease in food efficiency. The retarded gains were not attributable to reduced food intake or to the caloric inertness of the polyoxyethylene moiety of the test products. Marked changes in the intestinal tract and severe diarrhea were also observed when Myrj or Sta-soft was fed. Mortality data, data on organ weights and the gross pathology also indicated that the ingestion of these compounds was deleterious to the hamsters as compared to the data obtained on lard or lard plus celluflour.

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A Convenient Method for the Determination of Urea in Blood and Urine. (17704)

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The need for a convenient method of determining urea in blood and urine by direct Nesslerization following the action of urease has been recognized since 1917(1). There is no satisfactory way as yet to avoid cloudy solutions completely. Folin and Wu suggested that urea may be determined in blood and urine by estimating the ammonia formed after heating in acid solution at 150°C(1). Clark and Collip showed that urea is quantitatively converted to ammonia by heating for 10 minutes at this temperature(2), and Leiboff and Kahn devised a special pressure bottle in which the reaction may be conducted in an oil bath at 150°(3). Nevertheless, the method has not been adopted to any appreciable extent, since there was the possibility that ammonia might be formed from other nitrogenous constituents of urine. For more than a year we have obtained satisfactory results for the determination of urea by heating in a modern pressure cooker. The method described below is apparently not affected by other constituents of blood or urine.

Experimental. Two household pressure cookers were used in this study. One had a capacity of 4 quarts and a maximum pressure of 15 lb to the square inch (121°C). The other had a capacity of 12 quarts and a maximum pressure of 20 lb (126°C). Using 0.5 ml of 1 M·phosphoric acid to 2 ml of urea solution (0.01 to 0.2 mg·N), 90 minutes are required to decompose urea completely to ammonia at 121°, and 60 minutes are required at 126°. After cooling, the addition of Nessler's solution frequently produces cloudy solutions in less than 10 minutes when protein-free filtrates of whole blood are used. On the other hand, filtrates obtained from oxalated blood plasma or serum with 10 per cent sodium tungstate and 2/3 N·sulfuric acid remain clear for at least 30 minutes after the addition of Nessler's solution. Urine diluted with water also remains perfectly clear. However, urine must first be shaken with permutit to remove ammonium salts before the decomposition of urea. In addition, the concentration of urea may be adjusted to that of blood by diluting the filtered urine, after permutit treatment, according to a table(4),

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4. U. S. War Department, *Methods for Laboratory Technicians*, Tech. Manual No. 8-227, p. 107.