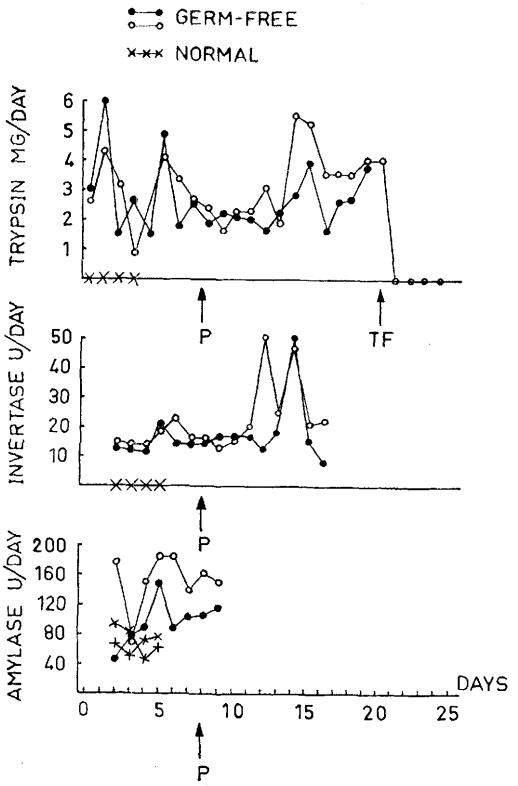

FIG. 1. Trypsin, invertase and amylase content of feces of germfree, ex-germfree and conventional rats. At P germfree animals were infected with sporeforming bacteria from the cecum of a conventional animal and at TF with suspension of feces from conventional rats.

Amylase content of the feces in the germfree rats was the same or slightly higher than that of the conventional rats. The occurrence of invertase in the feces of germfree animals agrees with the finding of carbohydrases in intestinal extracts from germfree rats(8). After infection of the germfree animals with sporeforming bacteria from the cecum content of a conventional animal, no decrease occurred in the enzymatic contents of the feces but rather sharp fluctuations were noticed in invertase and trypsin activity. This might be connected with the diarrhea that is a constant symptom in infection of germfree animals with certain intestinal bacteria. After superinfection with full intestinal flora of the conventional animals, tryspin content of the feces decreased to the zero values of the conventional rats within 24 hours.

Conclusions. Results indicate that autodigestion is not the main pathway for normally occurring inactivation of trypsin and invertase. The results infer that one or several of the normal microbiological inhabitants of the intestines are responsible for normally occurring inactivation of the digestive enzymes of intestinal contents.

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Effect of Dietary Fat on Excretion and Deposition of C^{14} from Cholesterol-4-C^{14} in Rats.* (25175)

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The relationship between serum cholesterol levels and dietary factors has been studied in man and lower mammals for many years. Recently it has become important to ascertain

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TABLE I. Fecal Excretion of C^{14} by Rats Fed Crisco, Corn Oil, or Coconut Oil and Injected with Cholesterol-4-C^{14}.

Dietary fat	No. of animals	Fecal excretion of C^{14} (% of administered dose) At 4 days Mean	S.D.*	At 14 days Mean	S.D.*
Crisco	10	13.7	4.6	51.9	7.6
Corn oil	10	11.6	3.2	51.7	7.6
Coconut oil	7	14.4	7.9	52.9	11.8

* Stand. dev.

the fate of serum cholesterol in those instances in which serum concentration has been influenced by specific dietary alterations. Evidence that cholesterol lost from human serum when unsaturated fats are added to the diet is excreted in some form was obtained by Hellman and coworkers(1) and by Gordon *et al.* (2). Wilson and Siperstein(3,4) noted no consistent differences in total recovery of fecal C^{14} by rats injected with cholesterol-4-C^{14} when fed 20% corn oil or 20% lard diets. We have studied the fecal excretion of C^{14} and incorporation of C^{14} in tissue lipids of rats fed diets containing corn oil, coconut oil, or hydrogenated cottonseed oil and injected with cholesterol-4-C^{14}.

Materials and methods. Male Sprague-Dawley rats weighing 75-100 g were placed on a purified diet‡ containing 20% fat. The fats used were: hydrogenated cottonseed oil (Crisco, iodine No. 73), corn oil (Mazola, iodine No. 123), and coconut oil (iodine No. 10). The rats were paired by weight and those in the group receiving corn oil or coconut oil fed the amount of food consumed by the corresponding rat on the Crisco diet the preceding day. A solution of cholesterol-4-C^{14} containing 1 mg and 680,000 cpm per ml was prepared by the use of Tween-20 according to the method of Meier *et al.*(5). After the rats had been on the experimental diets for 2 to 3 weeks 0.5 ml of the cholesterol solution was injected into the external jugular vein. Feces were collected at 1, 2, 4, 6, 10, and 14 days. The animals were then killed by decapitation, livers removed and placed immediately in 10% alcoholic KOH. The heart, lungs, aorta, and kidneys of each animal were pooled and treated as one sample with alcoholic KOH. After hydrolysis the unsaponifiable material was extracted with petroleum ether. Fecal samples were extracted using the method outlined by Siperstein and coworkers(6). Radioactivity determinations were made on all extracts by gas flow counting of direct mounts prepared according to the method of Entenman *et al.*(7). Corrections were applied for background and self-absorption. Serum total cholesterol determinations were done by the method of Sperry and Webb(8).

‡ Vitamin-free casein, 250 g; fat, 200 g; cod-liver oil, 20 g; sucrose, 480 g; vitamin supplement mixture with sucrose, 21 g (this mixture contained choline hydrochloride, 0.5 g; thiamine hydrochloride, 2.5 mg; pyridoxin, 2.5 mg; inositol, 1 g; calcium pantothenate, 30 mg; riboflavin, 5 mg); salt mixture (Mendel, Hubbell and Wakeman), 31 g.

Results. The per cent of administered dose of C^{14} excreted in feces by the 3 groups of rats is given in Table I. Similar quantities were excreted by the 3 groups at all time periods studied and approximately 52% of the administered dose was excreted in 14 days.

Analysis of C^{14} in extracts of total nonsaponifiable material in liver and in a pooled sample of heart, lungs, aorta, and kidneys revealed no significant differences in the 3 groups of rats (Table II).

No significant difference in serum cholesterol values of the 3 groups of rats resulted from feeding the various fats for a period of 4 to 5 weeks.

Discussion. These studies indicate that after 4 to 5 weeks on a purified diet containing 20% fat as hydrogenated cottonseed oil, corn oil, or coconut oil there is no difference in amount of C^{14} excreted in feces or found in livers or in a pooled sample of heart, lungs, kidneys and aorta in rats injected with cholesterol-4-C^{14}. In man there appears to be increased excretion of cholesterol derivatives

TABLE II. Amount of C^{14} in Tissues of Rats Fed Crisco, Corn Oil, or Coconut Oil and Injected with Cholesterol-4-C^{14}.

Dietary fat	% of administered dose Liver Mean	S.D.	Pooled organs* Mean	S.D.
Crisco	2.31	.50	1.86	.41
Corn oil	2.80	.65	1.85	.27
Coconut oil	2.21	.53	1.69	.41

* Heart, lungs, aorta, and kidneys.

when unsaturated fats are added to the diet (1,2), but Wilson and Siperstein(3,4) found no consistent difference in fecal excretion of C^{14} by rats fed 20% corn oil or 20% lard and injected intravenously with cholesterol-4-C^{14}. Both groups, however, excreted a larger amount of injected C^{14} than did rats fed a fat-free diet. The amount of C^{14} in non-digitonin-precipitable sterols was greatly increased in animals on the corn oil diet as compared to rats receiving either no fat or a 20% lard diet. In rats fed these fats at 30% of the diet the latter observation was confirmed and in addition, it was noted that in 4 out of 5 groups total C^{14} recovery in feces was greater in rats receiving corn oil. In our experiments using 20% fats in the diet the rats excreted about 52% of administered C^{14} in 14 days. This compares favorably with data of Wilson and Siperstein(3,4) in the experiments using fat at 20% of the diet. Fractionation of the C^{14} excreted in the feces was not included in our experiment. It is apparent that in these rats the effect of kind of fat fed is on type of sterol excreted rather than on total amount.

In studies of the influence of dietary fat on serum cholesterol levels it is necessary to ascertain the possible simultaneous effect of the diet on tissue deposition of cholesterol. Alfin-Slater *et al.*(9) found an accumulation of cholesterol in livers and adrenals of rats on an essential fatty acid-deficient diet with a corresponding decrease in serum cholesterol levels. Addition of essential fatty acids to the diet caused a reversal of this situation. The data presented in Table II indicate that under the conditions of our experiments varying the type of fat in the diet for a 4- to 5-week period did not influence amount of C^{14} deposited in livers or pooled organs (heart, lungs, aorta, and kidneys) after intravenous injection of cholesterol-4-C^{14}.

Summary. The influence of a purified diet containing 20% fat as hydrogenated cottonseed oil, corn oil, or coconut oil on fecal excretion and tissue deposition of C^{14} has been studied in rats injected with cholesterol-4-C^{14}. There was no significant difference between the 3 groups in amount of C^{14} excreted in feces or deposited in liver and in pooled internal organs (heart, lungs, aorta, and kidneys).

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Polarographic and Biologic Activities of N-Substituted Maleimides.* (25176)

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Friedman, Marrian and Simeon-Reuss(1) discovered that the maleimide molecule has antimitotic properties and forms –SH adducts readily. Recently(2) N-ethylmaleimide has been found to react with a number of thiols and thiol esters and the reaction

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