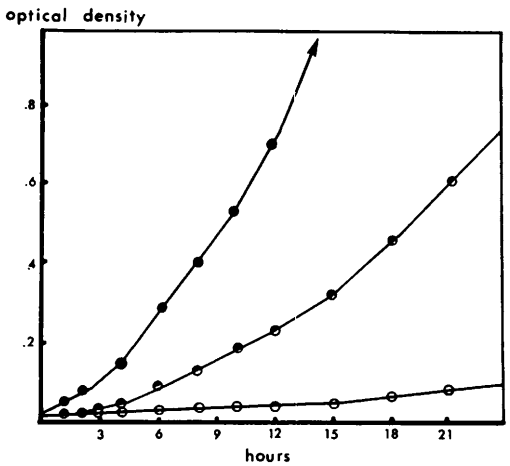

FIG. 1. Growth response of *C. stellatoidea* in complex glucose medium with up to 100 μg 6-mercaptopurine/ml (●), in minimal medium with (○) or without (◓) 50 μg 6-mercaptopurine/ml. Turbidity was measured at 625 mμ.

action of 6-mercaptopurine was partially overcome by adenine and hypoxanthine and less effectively by xanthine.

Discussion. The structure of 6-mercaptopurine resembles those of the 6-substituted purines adenine and hypoxanthine; evidence for competitive inhibition comes from the ability of adenine and hypoxanthine to restore growth and inductive capacity to 6-mercaptopurine inhibited cells. The ribotide, however, rather than the free purine, may be the active form(15,16). Both adenine and hypoxanthine are precursors for adenosine triphosphate, which also protects cells from the deleterious effects of 6-mercaptopurine.

Summary. The antitumor agent 6-mercaptopurine suppresses growth and induced enzyme formation by *Candida stellatoidea.* These inhibitory effects are substantially reversed by adenine and hypoxanthine.

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Effect of Dietary Fat on the Disposition of Cholesterol-4-C^{14} in Rats.* (27974)

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The hypocholesteremic effect of dietary unsaturated fat has stimulated interest in the fate of cholesterol disappearing from the serum. Hellman and coworkers(1) and Gordon *et al.*(2) found in humans that the cholesterol which disappeared from the serum after feeding unsaturated fats was excreted in the feces in some form. Bronte-Stewart *et al.*(3) reported that a fall in serum cholesterol level attributable to changes in dietary fat was accompanied by an increase of bile acids in feces. In rats fed diets contain-

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ing 30% lard or 30% corn oil for 1-16 days, Wilson and Siperstein(4) were unable to demonstrate any effect on excretion of cholesterol or cholesterol-C^{14} end products in the bile. In both short and long-term feeding experiments these workers found a greater fecal excretion of non-digitonin precipitable neutral sterols in rats fed corn oil than in those fed lard(5,6). Anderson *et al.* (7) found no significant differences in amount of C^{14} excreted in the feces or deposited in pooled internal organs in rats fed diets containing 20% fat as hydrogenated cottonseed, corn, or coconut oil for 2 to 3 weeks prior to intravenous injection with cholesterol-4-C^{14}.

In this investigation, studies reported by Anderson *et al.*(7) have been extended to include feeding periods of 30 to 76 days and analyses of individual organs.

Materials and methods. Male Sprague-Dawley rats weighing 125-175 g were allowed to eat *ad lib.* purified diets described previously(7) containing 25% protein, 48% sucrose, and 20% fats as hydrogenated cottonseed oil (Crisco), corn oil (Mazola), or coconut oil. Thirty days after initiation of the experimental diets and at various intervals thereafter, one rat from each group was injected intravenously with 1 ml of cholesterol-4-C^{14} solution containing 1 mg of cholesterol and 1.2×10^6 cpm(7). Following injection, feces were collected for 6 days, the animals killed by decapitation, and organs from each animal stored at −20°C until analyzed.

Analyses were performed as outlined previously(7). In addition, analysis of C^{14} activity was made on ethyl ether-extractable (bile acid-like) materials obtained after autoclaving samples at 15 lb pressure for 3 hours (5). Prior to this treatment, hydrolysis and petroleum ether extraction were used to effect separation of sterols from bile acids. Autoclaving was necessary for complete liberation of conjugated bile acids so they could be extracted with ethyl ether. Radioactivity was determined with a Packard Tri-Carb liquid scintillation spectrometer. All samples were checked for quenching and the indicated corrections made.

TABLE I. Body Weights.

Group No. and dietary fat	Initial wt	Wt gained
	g	
I. Hydrogenated cottonseed oil	168 ± 15	219 ± 27
II. Corn oil	165 ± 13	209 ± 36
III. Coconut oil	169 ± 13	175 ± 22

Values mean ± S.E. for all time periods.
6 rats per group.

Results. No consistent differences in food intake were noted in the 3 dietary groups. Initial body weights and gain in weight are given in Table I.

The amount of C^{14} found in the feces and intestinal contents is given in Table II. This is expressed as an average per cent of administered dose ± standard error for all time periods studied. A greater excretion of C^{14} in feces was observed in animals maintained on the hydrogenated cottonseed oil than on the other 2 diets. This is also seen in excretion data for individual animals (Fig 1). An increase in C^{14} excretion by all groups was observed as animals were maintained on diets for increasing periods of time. A slightly larger amount of C^{14} was found in the intestinal contents of animals fed corn oil and coconut oil than those fed hydrogenated cottonseed oil (Table II).

Fractionation of the fecal C^{14} activity into materials petroleum ether-extractable and ethyl ether-extractable after autoclaving showed that about half of the activity remained in the petroleum ether-extractable portion.

The percentage of activity in the petroleum ether fraction precipitable by digitonin tended to be lower in the coconut oil group than in the other two.

The amount of cholesterol excreted in the feces is shown in Fig. 2. Significantly smaller amounts of cholesterol were excreted by the coconut-oil fed rats than by those of the other 2 groups at all time periods.

Total cholesterol levels of livers at time of sacrifice are shown in Fig. 3 and summarized as averages in Table III. In the last 2 time intervals, cholesterol levels in livers of rats fed corn oil were lower, but not significantly so.

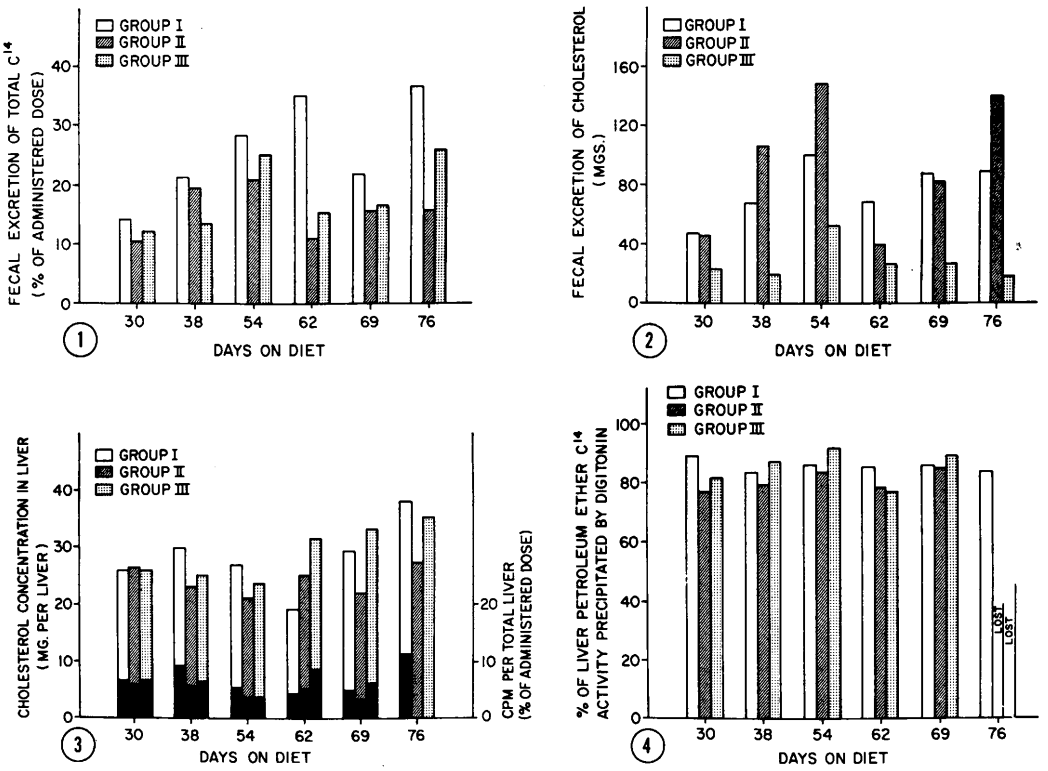

FIG. 1. Fecal excretion of total C^{14} expressed as % of administered dose. Group I, hydrogenated cottonseed oil; group II, corn oil; group III, coconut oil.

FIG. 2. Fecal excretion of cholesterol determined by Liebermann-Burchard colorimetric analysis after alkaline hydrolysis and precipitation with digitonin. Groups as in Fig. 1.

FIG. 3. Total cholesterol of livers as determined by Liebermann-Burchard colorimetric analysis after alkaline hydrolysis and precipitation with digitonin. Blackened areas are total C^{14} counts per liver as % of administered dose. Groups as in Fig. 1.

FIG. 4. Per cent of C^{14} activity of liver petroleum ether extracts precipitated by digitonin. Groups as in Fig. 1. 76 day samples of groups 2 and 3 lost.

Total C^{14} activity in livers of animals at sacrifice followed the same pattern as total cholesterol. Although animals of group 2 usually had the smallest amount of C^{14} (Table III, Fig. 2), the differences were not statistically significant. Only 2 to 10% of the total radioactivity of livers was found in bile acid-like material with no significant differences between groups. From 76 to 91% of the C^{14} activity present in petroleum ether

TABLE II. C^{14} Excretion in Feces and in Intestinal Contents of Rats Fed Diets Containing Various Fats.

Group No. and dietary fat	C^{14} in feces: % of administered dose	C^{14} in feces: % of activity in petroleum ether	C^{14} in feces: % of petroleum ether activity in digitonin ppt.	C^{14} in intestinal contents: % of administered dose
I. Hydrogenated cottonseed oil	26.2 ± 3.5	38.6 ± 2.4	69.4 ± 1.4	6.2 ± 1.0
II. Corn oil	15.5 ± 1.8	45.1 ± 5.4	72.4 ± 1.4	9.4 ± .8
III. Coconut oil	18.1 ± 2.4	32.6 ± 2.3	63.7 ± 3.3	8.7 ± 1.0

Values mean ± S.E. for all time periods.
6 rats per group.

TABLE III. Total Cholesterol and Total C^{14} in Livers of Rats Fed Diets Containing Various Fats.

Dietary group	Mg cholesterol per g liver	Mg cholesterol per total liver	Total C^{14} per liver (% of administered dose)	% of pet. ether extractable activity precipitated by digitonin
I. Hydrogenated cottonseed oil	1.94 ± .12	28.1 ± 2.5	7.0 ± 1.1	84.4 ± .4
II. Corn oil	1.89 ± .22	23.2 ± 1.1	4.9 ± .5	81.8 ± 2.0
III. Coconut oil	2.06 ± .17	29.1 ± 1.9	6.3 ± .7	84.5 ± 2.1

Values mean ± S.E. for all time periods.
6 rats per group.

extracts of livers was precipitable by digitonin.

Total radioactivity of organs of the different experimental groups was the same (Table IV). No correlation was evident between amount found and time of sacrifice after injection of C^{14}-cholesterol.

Neither serum cholesterol concentration nor serum C^{14} activity was affected by variation of the type of fat in the diet of these animals.

Discussion. The most impressive observation made as a result of these studies was the minimal effect which alteration of the type of dietary fat had on the disposition of intravenously administered C^{14}-cholesterol in the rat. Results obtained in rats kept on diets for 30 to 76 days are similar to those reported by Anderson *et al.*(7) for rats kept on similar diets for 14 to 21 days. In neither study was there an effect due to type of dietary fat on plasma concentration of cholesterol. Similarly, Olson, Jablonski, and Taylor(8) have reported that feeding of butterfat, lard, and corn oil at 40% of the diet for 3 weeks had no effect upon serum cholesterol in rats.

The only statistically significant differences noted between groups of rats on different dietary fats were a greater C^{14} excretion by the hydrogenated cottonseed oil-fed animals and a smaller amount of cholesterol excretion by the coconut oil-fed rats. The reason for the higher C^{14} activity excretion by the hydrogenated cottonseed oil-fed rats is not apparent. The measurement includes unchanged C^{14}-cholesterol plus all labeled metabolites extractable from feces. Only about 40% of the activity was extractable in petroleum ether (presumably cholesterol). The amount of cholesterol excreted in the feces as measured in this study includes all petroleum ether-extractable materials which precipitated with digitonin and gave a Liebermann-Burchard color. A significant portion of the cholesterol excreted in the feces may have been endogenous in origin and the smaller excretion of cholesterol by coconut oil-fed rats may have been due to an effect of dietary fat on endogenous synthesis of cholesterol. Avigan and Steinberg(9) reported that more acetate-1-C^{14} activity was incorporated into the liver and serum cholesterol of corn oil-fed rats than in coconut oil-fed or control animals. The presence of slightly greater amounts of C^{14} activity in the intestinal contents of corn oil-fed and coconut oil-fed rats than in hydrogenated cottonseed oil-fed rats would probably not affect the results obtained for feces since the

TABLE IV. Total C^{14} Activity in Organs of Rats Fed Diets Containing Various Fats.

Dietary group	Total C^{14} activity (% of administered dose)				
	Intestine	Lungs	Spleen	Kidneys	Heart
I. Hydrogenated cottonseed oil	2.7 ± .4	1.5 ± .3	.73 ± .04	1.1 ± .1	.50 ± .05
II. Corn oil	3.4 ± .6	1.8 ± .1	.76 ± .04	1.0 ± .0	.47 ± .02
III. Coconut oil	3.4 ± .7	1.9 ± .2	.70 ± .07	1.0 ± .1	.46 ± .04

Values mean ± S.E. for all time periods.
6 rats per group.

C^{14} activity would be subject to absorption and re-secretion several times before reaching the point of actual fecal excretion. This constant absorption and re-secretion of cholesterol and bile acids may form part of the mechanism controlling hepatic synthesis, esterification, and degradation of cholesterol.

An attempt was made in these studies to determine whether any gross differences in the nature of C^{14} metabolites excreted by the different groups could be demonstrated. It is recognized that the procedure used for separation of bile acids from cholesterol or other sterols does not result in completely separated fractions. Within the limitations imposed by this technic, no statistically significant differences were observed between the various groups. However, slightly greater amounts of petroleum ether extractable C^{14}-substances were found in feces of the groups fed hydrogenated cottonseed oil and corn oil than were found in the group fed coconut oil and these paralleled the amount of digitonin-precipitable C^{14} (cholesterol) in the petroleum ether fraction. Wilson and Siperstein(5) observed an increase in non-digitonin precipitable fecal sterols in rats fed corn oil compared to those receiving lard or a fat free diet.

Although animals of each group have been treated together in this discussion, the rats were killed at intervals from 30 to 76 days to determine if any trend (with time) could be observed in any of the measurements made. The most suggestive of these was the increasing amount of fecal C^{14} and of fecal cholesterol in the group fed hydrogenated cottonseed oil and to some extent in the other 2 groups.

Summary. Rats were maintained for 30 to 76 days on a purified diet containing 20% fat as hydrogenated cottonseed oil, corn oil, or coconut oil and then injected intravenously with a tracer dose of cholesterol-4-C^{14}. In the 6 days following injection higher excretion of C^{14} was observed in animals maintained on hydrogenated cottonseed oil. Of the activity extracted by petroleum ether after saponification, a lower percentage was present in the digitonin-precipitable fraction in animals fed coconut oil. Smaller amounts of cholesterol were excreted by the coconut oil-fed rats. No consistent differences were observed in total C^{14} activity of various organs of the different dietary groups at any time period. Neither serum cholesterol nor serum C^{14} activity was affected by variation of the type of dietary fat given these rats.

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Multiple Forms of Glutamic-Oxalacetic Transaminase in Tissues. (27975)

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Glutamic-oxalacetic transaminase (GOT) is an enzyme which is widely distributed in both animal and plant tissues(1) and is of considerable significance due to its role in glutamate oxidation(2). In addition, the elevation in serum levels of this transaminase in certain disease states(1) makes the study of this enzyme of particular importance. Recently,