Effect of Dietary Fat and Fatty Acid on Fecal Excretion of a Calcium Oleate Phosphate Complex.* (22560)

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Kim and Ivy(1) and Pihl(2) reported that rats fed an oleic acid diet excreted large amounts of phospholipid in their feces. Palmitic acid and corn oil were found to have a mild stimulatory action on phospholipid excretion(1). These authors did not analyze the lipid but assumed that it was phospholipid since it contained phosphorus and could be precipitated from ether solutions with acetone. In the course of work on sterol absorption in these laboratories(3) this material was also encountered in fecal extracts when rats were fed oleic acid diets, and it became of interest to characterize this lipid(4). Chemical and infra-red analysis revealed that the "phospholipid" was a salt containing 2 atoms Ca^{++}, 1 mole $HPO_4^{=}$, and 2 moles oleic acid. It had a pK of 6.1 and was readily dissociated by acid, but was not cleaved by base. A lipid of this composition has not been previously reported and it is of particular interest in that it contains phosphorus and the solubility properties of some phospholipids. P^{32} experiments indicated that the lipid was formed predominantly in the gut from dietary calcium, phosphorus, and oleic acid. The following study was conducted to explore further the formation of this complex in the intestine and also to determine what effect its formation would exert on fat absorption.

Methods and materials. Rats weighing 175-200 g were housed in individual cages arranged for quantitative collection of feces. The diet in slight excess of the daily requirement was weighed and placed in food cups. The diet remaining the following day was weighed and the food intake calculated by difference. The diet consisted of the following: 20% casein, 24% corn starch, 24% glucose, 25% fat or fatty acid, 2% methyl cellulose, 5% Hubbell-Mendel-Wakeman salt mixture, and adequate amounts of crystalline vitamins. An additional 25% carbohydrate was added to the control diet without fat. The rats were divided into 6 groups and each received a different fat or fatty acid in their diet as indicated in Table I.

The diets were fed for 8 days, the feces collected daily, and pooled in each group. The feces of each group were ground and extracted 4 times with boiling 2:1 chloroform-methanol. The extract was washed with water, dried over Na_2SO_4, and filtered. An aliquot of this original extract was taken for analysis and then the remainder of the extract was concentrated to 200 ml in an atmosphere of nitrogen. The phosphorus-containing lipid was precipitated from the chloroform extract once with acetone and then 4 times with methanol. Total lipids were determined on the original extract and on subsequent precipitations by drying at low temperature to constant weight. The amount of the phosphorus-containing lipid present in feces is based on the analysis and weight of the lipid after 5 precipitations. Phosphorus, calcium, and fatty acid content of the lipid were determined on the original extract and at each precipitation as previously described (4). Apparent fat absorption was determined from the dietary fat intake and excretion values, taking the control excretion of the fat-free group into account.

Results. The results are shown in Table I. The control group (F-1) excreted only a trace of lipid-P in their feces. A comparison of the dietary fats shows that corn oil and olive oil stimulated the formation of the complex salt, whereas hydrogenated soybean oil had virtually no effect on lipid-P excretion. Corn oil and olive oil were well absorbed, 95.7% and 97.1% respectively, and hydrogenated soybean oil poorly absorbed, 56.9%. In the case of corn oil and olive oil, the phosphorus-

* This investigation was supported in part by a research grant, H-1897, from the Public Health Service.

TABLE I. Effect of Dietary Fat and Fatty Acid on Fecal Excretion of a Calcium Oleate Phosphate Complex.

Group	No. rats	Dietary fat (25%)	Fat intake per day per rat, mg	Fecal lipid excretion/day/rat — Total lipid, mg	Lipid P,* mg	COP,† mg	Non-COP lipid, mg	Apparent fat absorption,‡ %
F-1	7	None	0	26	Trace	Trace	26	
F-2	7	Hydrogenated soybean oil	2433	1074	"	"	1074	56.9
F-3	7	Palmitic acid	2433	450	.57	7	443	82.6
F-4	6	Corn oil	2490	133	1.00	17	116	95.7
F-5	7	Olive "	2477	98	.90	10	88	97.1
F-6	7	Oleic acid	2491	774	12.52	658	116	70.0

* Determined on original chloroform-methanol extract.
† Calcium oleate phosphate complex. Determined by weighing after 5 pptns. by methanol.
‡ Taking control excretion (Group F-1) into account.

containing lipid accounted for approximately 10% of the total lipid excretion. Of the dietary fatty acids, palmitic and oleic, oleic greatly increased lipid-P excretion. This excretion was above palmitic or any of the fats tested. Palmitic acid had only a slight stimulatory action (.57 mg/day), whereas oleic increased lipid-P excretion to 12.52 mg/day or 658 mg day of the lipid. This increase accounted for 85% of the total lipid excreted and its formation greatly impaired the absorption of oleic acid (70.0%).

Discussion. The present study has confirmed and extended the observations of Kim and Ivy(1). A comparison of the effect of the different fats on the formation of the calcium fatty acid phosphate complex reveals that the unsaturated fats, corn oil, and olive oil, were more effective in formation of this lipid than the saturated fat, hydrogenated soybean oil. Also, of the fatty acids tested, oleic was far more effective in stimulating formation of the complex than palmitic acid. In the previous study(4) in which the lipid was characterized, it was found that essentially all of the fatty acid was oleic. Thus, this acid would appear to be relatively specific for formation of this complex. Corn oil and olive oil would contribute a small amount of oleic acid during their hydrolysis in the intestine for formation of this lipid complex, whereas the hydrogenated fat would contribute very little oleic acid.

It is apparent that oleic acid absorption is definitely impaired due to formation of this lipid; there being only 70% absorption in the present experiment. The absorption of this acid was even less than palmitic acid (82.6%) which is unusual since unsaturated fatty acids have been shown to be better absorbed than the saturated ones. However, the conditions utilized for determining oleic acid absorption have generally been based on single feedings of the fatty acid alone. It would appear that the feeding of the fatty acid in a diet would more nearly approach physiological conditions of absorption. It is also apparent that dietary phosphorus and calcium absorption are impaired when an oleic acid diet is fed. Based on the dietary intake and excretion of calcium and phosphorus, it was calculated that 50% of the calcium and 50% of the phosphorus were lost in the feces as part of the calcium fatty acid phosphate complex.

At present, it is not known how this lipid is formed in the intestine and what, if any, role it has in fat absorption. Since it is formed predominantly with free unsaturated fatty acid in the intestine, it would appear that very little free unsaturated fatty acid is formed during the digestion of corn oil and olive oil.

Summary. The excretion of a calcium oleate-phosphate lipid complex in the feces of rats was studied under various dietary conditions. Diets containing 25% of either olive oil, corn oil, hydrogenated soybean oil, palmitic acid or oleic acid were compared with a fat-free basal diet. Oleic acid was approximately 70 times more effective in stimulating formation of lipid than either corn oil or

olive oil. Palmitic acid had a very slight effect, whereas hydrogenated soybean oil had no effect. The absorption of oleic acid was much lower than that of palmitic acid, corn oil or olive oil, perhaps due to the formation of the lipid. The significance of the fecal lipid is discussed.

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Received June 11, 1956. P.S.E.B.M., 1956, v92.

Life Span of the Duck and Chicken Erythrocyte as Determined with C^{14}. (22561)

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Carbon 14 labeled glycine has been widely used to determine the life span of the non-nucleated erythrocytes of man(1), and several of the lower mammals(2-4) but apparently has not been used to study the life span of the nucleated erythrocytes of the bird. Shemin(5) using N^{15} and Hevesy *et al.*(6) using P^{32} found the life span of the chicken erythrocyte to be 28 days. McConnell *et al.* (7) using Se^{75} to label the cells, determined the life span of the duck erythrocyte to be 11.7 days. This present study was undertaken to provide additional information on the erythrocyte life span of these 2 species by the use of C^{14} and to evaluate the applicability of this material in the measurement of the life span of nucleated erythrocytes.

Methods. Glycine-2-C^{14} was injected intravenously into the wing vein of the birds. Two adult female chickens were given 17 μc per kilo of body weight and 2 adult male ducks were given 35 μc per kilo. Two additional ducks were given the same dose intraperitoneally for studies of the rate of uptake. One half ml of blood was obtained at regular intervals after the initial injection. Samples containing 0.1 ml of packed cells were prepared by washing the cells 3 times with saline and precipitating the protein with 10% trichloroacetic acid. The precipitate was washed and plated onto a 1 inch copper planchet and counted in a gas flow proportional counter.

Results. The radioactivity of the red cell protein of the duck and chicken is shown as a function of time in Fig. 1. There was an unusually rapid uptake of the labeled carbon into the protein of the duck cells. This response was independent of the method of injection because the rapid uptake was also observed following intraperitoneal injection of the glycine. The character of the uptake curve and the double hump noted at the peak of the curve suggested that approximately 10% of the glycine α-carbon incorporation was in the red cells already in the peripheral circulation. The sigmoidal decay was typical of that observed in man and the mammals where only a relatively homogenous age group of cells forming in the marrow are labeled. We therefore assumed that the lower plateau and decay curve represent the labeling of the more or less homogenous age group forming in the marrow at the time of injection. An analysis of the curves gave a value of 44 days from the initial labeling until 50% of the cells were destroyed with half of the labeled cells destroyed in the interval $\pm$ 5 days. The mean survival time, which represents the average time during which a given cell may exist in the peripheral circulation, is ordinarily computed as the interval between the appearance and disappearance of 50% of the labeling. If we ignored the unusually rapid uptake the value was 42 days. If we assumed that the