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Incorporation of C^{14}-Acetate into Intestinal Fatty Acids of Rats with Cannulated Bile Ducts.* (22230)

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Incorporation of C^{14} from acetate into intestinal fatty acids has been demonstrated in experiments of short duration by various investigators(1-3). Evidence is available which indicates that the labeled intestinal fatty acids are synthesized independently of the liver fatty acids(2) and probably by the intestine itself(3,4). However, the possibility of transport of significant amounts of highly radioactive fatty acids from liver to intestine via the bile has not been investigated. This pathway was suggested to us by the findings of Gould *et al.*(5), that significant amounts of synthesized liver cholesterol are transported via the bile. The experiments described in this paper were designed to ascertain the role of bile in the acquisition of labeled long chain fatty acids by the intestine after administration of radioactive acetate.

Methods and materials. Adult male Sprague-Dawley rats weighing 200 to 300 g were used, and animals of the same weight were paired in each experiment. The bile duct of one rat was cannulated with polyethylene-50 tubing just distal to its bifurcation, and the cannula led to the outside of the body through an incision immediately below the right costal margin. A sham operation was performed in the control animal. Infection was not a problem and recovery usually occurred in 7 to 9 days. The sham-operated animal was pair-fed with the cannulated rat. During the first 2 days post-operatively there was low food consumption which led to a 5-10% decline in body weight. Subsequently, food consumption returned to normal and body weight remained at a constant level or showed a slight gain. When the rats had recovered, they were injected intraperitoneally with a tracer dose of radioactive acetate (1 ml of solution containing 3 mg of $CH_3C^{14}OO$ Na and 10 μc of activity) and placed in a cage designed to hold animals immobile(6). Water was given *ad libitum* but food was withheld after C^{14} administration. Three pairs of animals were allowed to metabolize the radioactive acetate for 5 hours; 2 pairs were killed 30 minutes after injection. Samples of bile were collected in the 5-hour experiments at 30-minute intervals for the first hour and at hourly intervals thereafter. A 0.025 ml aliquot of each bile sample was dried on a planchet and counted as such to determine the relative activity of the whole bile. In the 30-minute experiments 0.025 ml samples were taken at 5-, 15-, and 25-minute intervals. No corrections for self-absorption were applied to these measurements. The liver, intestine, bile and combined contents of cecum and intestine were taken for chemical and

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TABLE I. Specific and Total Activity of Intestinal Fatty Acids of Cannulated Rats and of Sham-Operated Controls after Administration of $CH_3C^{14}OONa$.

Time killed after inj. of C^{14}-acetate	Specific activity, c/m/mg fatty acid		Total activity, c/m/100 g rat	
	Cannulated	Control	Cannulated	Control
5 hr	109	50	3600	4600
5 "	160	109	8500	4100
5 "	73	50	4600	3500
30 min.	61	49	4700	3200
30 "	52	21	4350	1750

radioactivity analyses of the total fatty acids. Chemical analyses were done as described previously(7). To each sample of bile was added 10 mg of inert palmitic acid before saponification that adequate material might be available for analysis. Correction for this addition was made in both chemical and radioactivity analyses. For determination of radioactivity fatty acids were oxidized to carbon dioxide by the method of Thorn and Shu(8) and samples counted as barium carbonate to a total of 3000 counts in windowless flow counter.

Results. The data presented in Table I show that the total activities of intestinal fatty acids of cannulated rats were similar to those of sham-operated controls. In fact, the values obtained in cannulated animals were in most cases slightly higher than those of controls. The specific activity of intestinal fatty acids was definitely higher in the cannulated group.

Analyses of biliary fatty acids revealed that specific activity values reached a maximum in one-half to one hour after injection and remained almost constant or declined slowly thereafter. The average specific activity and total activity of biliary fatty acids for 3 cannulated rats are shown in Table II. It can be seen that in each case total activity obtained in biliary fatty acids represented only 6 to 16% of that found in the intestine itself.

TABLE II. Radioactivity of Biliary and of Intestinal Fatty Acids of Cannulated Rats after C^{14}-Administration.

Rat No.	Specific activity, c/m/mg fatty acid		Total activity, c/m	
	Biliary fatty acids	Intestinal fatty acids	Biliary fatty acids	Intestinal fatty acids
1	93	109	1600	9950
3	53	160	400	15500
5	97	72	900	12800

The specific activity of whole bile reached a maximum in one hour or less but declined at a faster rate than that of the biliary fatty acids.

The amount and radioactivity of fatty acids of combined intestinal and cecal contents were compared for normal and cannulated rats (Table III). In 4 of the 5 pairs studied the fecal material from cannulated animals contained more fatty acid than did that from controls. In 3 of the 4 experiments in which data are available the specific activity of fecal fatty acids from cannulated rats was lower than that from controls. However, total activity of the fecal fatty acids was similar for the two groups.

There was no significant effect of deviation of bile on either specific or total activity of the liver fatty acids.

Discussion. These experiments indicate that bile does not transport significant amounts of liver-biosynthesized fatty acids to the intestine. This knowledge is important in studying the possible sources of highly labeled intestinal fatty acids observed after administration of C^{14}-acetate, and the findings are consistent with the hypothesis that intestinal synthesis from acetate is the source of labeled long chain fatty acids.

A greater amount of fatty acid was found in the fecal material of cannulated rats than was found in controls. Mild steatorrhea had been observed in cannulated rats 4 to 5 days postoperatively. The increased fecal fat content was no doubt due partly to incomplete absorption of ingested fat. However, increased secretion of endogenous fat in bile-cannulated animals has also been reported (9). The lower specific activity of fecal fatty acids of cannulated rats is consistent with the presence of more fat in the intestinal lumen acting to dilute labeled fatty acids synthesized from radioactive acetate. It should be expected that increased secretion of endogenous fat in the cannulated animals would

TABLE III. Radioactivity of Fatty Acids of Combined Intestinal and Cecal Contents of Cannulated and Sham-Operated Rats after Intraperitoneal Injection of C^{14}-acetate.

Time killed after inj. of C^{14}-acetate	Total fatty acids: Amt of fatty acids, mg C*	S†	Specific activity, c/m/mg fatty acid C	S	Total activity, c/m C	S
5 hr	39.0	102.0	45.6	30.2	1780	3070
5 "	74.8	17.6	13.7	21.0	1025	370
5 "	53.0	22.6	11.8	20.3	625	460
30 min.	31.1	11.8	7.3	12.0	227	142
30 "	45.5	17.6	6.0	—	272	—

* C = Cannulated rat. † S = Sham-operated rat.

also result in increased total radioactivity in the fecal fatty acids. This was observed in 3 experiments but the opposite result was obtained in a fourth experiment.

The intestinal fatty acids of cannulated rats had a higher specific activity than those of sham-operated controls. The reason for this difference is not known.

Summary. 1. Deprivation of bile flow to the rat intestine by means of cannulation of the bile duct did not affect the ability of the intestine to accumulate normal amounts of highly labeled fatty acids after administration of C^{14}-acetate as shown by comparison with sham-operated, pair-fed controls. 2. Total radioactivity in the biliary fatty acids amounted to only 5-16% of the amount found in the intestinal fatty acids in the time periods studied. 3. Fatty acids of combined intestinal and cecal contents of cannulated animals contained more fatty acid but of a lower specific activity than those of control rats.

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Additional Serotypes of the APC Virus Group. (22231)

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Six serotypes of the adenoidal-pharyngeal-conjunctival (APC) group of viruses have been described(1,2). The purpose of this communication is to report eight additional serotypes from both human and simian sources which have been isolated in this and other laboratories.

Methods for characterization and identification of APC viruses. For purposes of classification, the cardinal attribute of the APC virus group is the group specific complement fixing antigen. This antigen is not shared with other known viruses, and complement fixing antibody against it is not produced by other known infections(1-3). The procedure presently employed for determining the presence of this antigen in candidate strains is as follows: using the complement fixation procedure previously described(2), the titer of the candidate antigen is determined by testing with a pool of human convalescent serums having high titer complement fixing antibody to the group antigen. Four units of the candidate antigen is then used in tests for antibody in paired serums of at least 4 persons, each known to have been infected with a different APC type, and each showing at least an 8-fold rise against the virus antigen