that repeated administration of thyrocalcitonin results in an inhibition of all phases of bone resorption.

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The Effect of Bile on Vitamin A Absorption in the Rat* (32630)

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It is generally accepted that after absorption long chain fatty acids are mainly converted to triglyceride in the intestinal mucosa and then leave the intestine by the lymphatic route (1, 2). Recent evidence suggests that in the absence of bile the partition of long chain fatty acids between the portal vein and lymph is altered to favor the portal route (3, 4) and this route may be used to a variable degree normally (5). We have now extended these observations using vitamin A as another model compound, for it is usually considered that absorbed vitamin A is esterified and also transported by the lymphatic route (6).

Material and Methods. Crystalline unlabeled vitamin A acetate, palmitate, and alcohol were a gift from Roche Products Limited, Basle. Standard solutions were prepared in *n*-hexane. Vitamin A acetate (Carbinol ^{14}C) (Radiochemical Centre, Amersham, England) had a specific activity of 2.92 mC/mole. The radiochemical purity by thin-layer chromatography (TLC) on silica gel G (cy-

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clohexane ether 4:1) was 98%. Standard solutions were prepared in benzene.

Butylated hydroxytoluene (BHT) was added to all solvents as an antioxidant. Standard solutions and crystallized material were kept under nitrogen in the dark in the deep freeze.

Experimental procedure. Two groups of 11 Sprague-Dawley rats weighing 250–350 gm were studied. Under ether anesthesia a U-shaped catheter was inserted into the thoracic duct below the diaphragm by the slightly modified method of Bollman (7). Through a gastrostomy a fine polythene feeding tube was placed in the gastric pouch. In 1 group of 11 rats, the bile duct was also cannulated as close as possible to the bifurcation of the hepatic duct and the bile drained through the anterior wall of the abdomen. The remaining group served as a control. The animals were then placed in a restraining cage and had access to a solution of glucose 5%, NaCl 0.9% and KCl 0.04%. After a 24-hour recovery period, the rats were fed 0.2 ml of a freshly prepared solution of corn oil containing ^{14}C-labeled vitamin A acetate with 500 μg of vitamin A carrier acetate, plus 1 mg of α-tocopherol. Lymph was then collected for 8 hours and the rats were killed under ether anesthesia. The lymph, liver, and entire gastrointestinal tract with its contents were homogenized separately in water and made up to volume. The tissue lipid was extracted with ethanol–hexane 1:2 (v/v) and the amount of radioactivity in the hexane phase determined in a liquid scintillation counter, quenching was assessed by the channels ratio method (8). The efficiency of this extraction procedure was over 90%.

Vitamin A was identified by TLC against known standards of vitamin A alcohol, acetate, and palmitate, performed in an atmosphere of nitrogen and in the dark to minimize oxidation. Separate bands of fluorescence were shown under ultraviolet light, then scraped off into Bray's solution and counted for radioactivity.

Results. Total recovery of ^{14}C-labeled material. The results are expressed as the percentage of the fed vitamin A acetate-^{14}C which was recovered from the lymph, animal tissues, and gastrointestinal contents extracted with ethanol–hexane. In the control animals the total recovery ranged between 46 and 88% with a mean of 63%, while in the bile fistula rats this was even lower, ranging from 19 to 88% with an average of 46.7% (Table I). To account for the poor recovery of labeled material from the extracted tissues, the lipids from the kidneys, adrenal, lungs, heart, spleen, blood, and urine were also extracted and both ethanol and hexane phases were counted for radioactivity. The amount of label thus recovered was negligible in the control animals, they could represent 10–16% of the absorbed vitamin A-^{14}C in the bile fistula rats.

TABLE I. Absorption and Lymphatic Transport of Vitamin A Acetate-^{14}C in Control and Bile Fistula Rats.

Eleven rats in each group; mean ± SEM.

	Control	Bile fistula	t	p
Total recovery (%) of lipid soluble ^{14}C	64.4 ± 4.5	48.0 ± 6.3	2.1	<.05
Absorption of vitamin A-^{14}C (%)	18.2 ± 3.0	0.84 ± 0.55	5.8	<.01
% of absorbed vitamin A in lymph	90.6 ± 2.6	51.7 ± 6.3	5.7	<.01
Lymph/liver ratio ^{14}C	17.7 ± 3.7	1.5 ± 0.43	6.2	<.01

Vitamin A acetate-^{14}C was then incubated in broth (Beef digest broth - Southern Group Laboratory, Lewisham) and also in broth to which was added a suspension of cecal contents of a rat, (24 hours at 37°C). The suspension was then extracted with ethanol–hexane 1:2 (v/v). The labeled vitamin incubation in broth alone was totally recovered in the hexane phase, whereas only 66–68% of vitamin A-^{14}C incubated with feces could be accounted for as lipid soluble compounds (Table II). Furthermore, thin-layer chromatography of the lipid extract of the incubation mixture containing rat cecal contents showed a series of polar compounds running in the front of the plate. This suggested that bacteria degraded vitamin A into nonlipid compounds.

Vitamin A acetate-^{14}C absorption. It is as-

TABLE II. Effect of Incubating Vitamin A Acetate-^{14}C with Rat Cecal Contents.

Recovery (%) of lipid soluble radioactivity after incubation in broth at 37°C for 24 hours.

Experiment	Control	+ Cecal contents
1	99	66
2	98	68

sumed that the amount of radioactivity recovered from the lymph and liver represents the bulk of the absorbed vitamin A-^{14}C in the rat. Vitamin A is poorly absorbed in the absence of bile (Table I); there is a mean absorption of 19.8% in the control animals and 0.3% in the bile fistula animals.

In the control animals the vitamin A-^{14}C recovered from the lymph represents 76–97% of the total absorbed vitamin, with an average of 90.8%. The recovery from the lymph of bile fistula rats varies from between 13 and 82%, with an average of 49.4% (Table I). The lymphatic system is the main route of transport for the absorbed vitamin A acetate from the gut in the normal, but can account for only 50% of transport of the absorbed vitamin in the absence of bile. The difference between the bile fistula animals and controls is further emphasized by calculating the ratio of radioactivity recovered in the lymph and the liver, they differ strikingly in the two groups. In the bile fistula rats, 6 of the 11 animals actually had more radioactivity in the liver than in the lymph.

Identification of the absorbed labeled material. The radioactive material extracted from the liver and the lymph from control and bile fistula animals was identified by TLC and found to be predominantly a long chain fatty acid ester of vitamin A. The lymph from the bile fistula rat did not contain sufficient amount of radioactivity to permit the identification of the labeled material.

Discussion. These results have confirmed the importance of bile in the absorption of vitamin A in the rat but demonstrated that small amounts can still be absorbed (9). In this respect vitamin A differs from long chain fatty acids or triglycerides, of which there can be up to 50% absorption of oleic acid in the absence of bile (5, 10). The relative ease with which vitamin A is changed by bacterial metabolites makes it difficult to use it in quantitative absorptive studies and probably accounts for the low recovery of vitamin A in the bile fistula animals where presumably bacteria converted more of the unabsorbed vitamin A into water soluble metabolites. In view of these effects the amount of ^{14}C recovered in tissue and lymph was used as an index of the absorbed material.

In bile deficient rats only 50% of the absorbed vitamin A could be recovered from the lymph and the lymph/liver ratios were strikingly modified. It is unlikely that this could be explained simply by the opening up of collateral lymphatic channels in the bile fistula animals and is more likely that it represents some transport by the portal route, and indeed by analogy with long chain fatty acids (11), the portal route may even be used normally as a minor pathway, although it is impossible to be sure of this from the data presented in this paper as smaller amounts in the normal animals could be due to lymphatic or lymphatico-portal collateral vessels. Unfortunately, the form of the vitamin A in the lymph of the bile fistula animals is unknown. It is possible that free vitamin A alcohol could pass through the cell and enter the extracellular fluid and become attached to plasma protein just as unesterified fatty acids can. If this occurred once the vitamin A alcohol reached the extracellular fluid one would expect it to pass with equal facility into the portal capillaries as into the lymphatic system: indeed, transport by the portal route might be favored in view of the far greater flow through the portal capillaries as compared with the lymphatic vessels.

Summary. Two groups of 11 Sprague-Dawley rats were fed vitamin A-^{14}C in corn oil, both had thoracic duct fistulas, one group in addition had bile fistulas. There was a poor total recovery of vitamin from the animal tissue and gastrointestinal contents of both groups of animals and this was less in the bile fistula animals where less vitamin A was absorbed. This is most likely explained by the degradation of lipid soluble vitamin A into water soluble metabolites by bacteria in the large intestine. In the control animals more radioactivity was recovered from the in-

testinal lymph than the liver and other tissues, suggesting that most of the absorbed vitamin A was transported by the lymphatic route. In the absence of bile, however, when the absorption of vitamin A was grossly impaired, there was a tendency for more to be recovered from the liver than the lymph, suggesting that an alternative route of transport of the absorbed vitamin was used.

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Fatty Acids of RBC Ghosts, Liver Mitochondria and Microsomes of Cold-Acclimated Hamsters* (32631)

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Fawcett and Lyman (1) and later Kodama and Pace (2) showed that cold acclimation produces changes in the levels of unsaturated fatty acids in white adipose tissues of hamsters. Recently Williams and Platner (3) studying whole homogenates of livers of cold acclimated hamsters also found that there were changes in the fatty acid percentage composition. The obvious question is: does this change occur solely in the hamster liver depot fat or are there also changes in the liver lipoprotein membranes, both those already present and those newly formed?

Chaffee Hoch, and Lyman (4) have found that prolonged cold-exposure affects even the appearance of mitochondrial pellets of hamster livers. In addition to the change in appearance, mitochondrial counts per gram of liver rise about 18–20% by the 9th week of cold acclimation at 2 ± 1°C. Since normal mitochondrial replacement no doubt also takes place throughout acclimation, a considerable portion of the liver mitochondria of such cold-acclimated hamsters can be expected to be newly formed. Similarly, Reynafarje and Chaffee (5) found a noticably larger liver microsomal pellet which differed in appearance in cold-acclimated hamsters. Therefore, during cold-acclimation, it is reasonable to suppose that lipoprotein membrane synthesis occurs in the liver, where it is known that percentage levels of total fatty acids also change (3).

Brock (6) has reported that the half-life of the red cells (RBCs) of both awake cold-acclimated, and control hamsters is about 38 days. Our cold-exposure period was 5–9 weeks, which would allow for the genesis of at least some new RBCs. However, since these originate from preformed hemocytoblasts which may be stored, it is obvious that longer acclimation should yield a higher percentage of newly formed RBCs, and thus more newly formed lipoprotein plasma material.

Therefore, we have made comparative studies by means of gas chromatography of

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