Intestinal Absorption of Micellar Lipid in Normal and Bile Deficient Rats[1] (35117)

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Much of the current work on intestinal absorption of lipids relates to the development and testing of the concept that the higher fatty acids are absorbed as soluble complexes with the bile acids. This concept was stated as follows by Verzar as early as 1936 (1): "The main action of the bile acids, glyo- and taurocholic acid, is not an emulsification, or a lipase activation, but a solution of the fatty acids, the products of lipase." Verzar referred to the soluble bile acid–fatty acid complexes as choleic acids. Currently they are referred to as micelles and are known to contain not only fatty acids but also monoglycerides which are now recognized as normal end products of pancreatic lipase action (2).

At the bile acid concentrations normally existing in the intestinal lumen (5–10 m*M*) somewhat less than 10 μmoles (3.5 mg) of monoglyceride or fatty acid/ml can exist in micellar form (2, 3), which means that if all dietary triglyceride (100 g or more/day in the human being) goes through this phase, the micelle turnover rate must be extremely rapid. The bile acids must also undergo rapid turnover with reutilization since the amount required to solubilize 100 g of lipid in this way greatly exceeds the pool size (2). The turnover of bile acids via the enterohepatic recirculation mechanism is well known (2). Verzar (1) also believed that the bile acids, after being freed from the choleic acid complex, remained adsorbed to the cell surface and were reutilized in that manner.

There has been some evidence to suggest that the bile acids play a role in the lipid transport phase by stimulating fatty acid esterification in the mucosa. In the bile fistula animal given fatty acids by stomach, smaller than normal fractions of absorbed fatty acids are recovered as triglycerides in the mesenteric lymph and larger than normal fractions are recovered as free fatty acids in the lymph and portal blood (4, 5). The present study deals with this question in particular and in general provides strong supportive evidence for the validity of the micellar concept.

Methods. Male Sprague-Dawley rats weighing 250–350 g were fasted 24 hr. They were then anesthetized with sodium pentobarbital and prepared with a variety of perfusion and drainage tubes as follows: a polyethylene tube (Clay Adams PE 50) in the thoracic duct above the cisterna chyli (6); a polyethylene perfusion tube (PE 50) through the stomach wall and threaded through the pylorus into the duodenum (a ligature was then applied around the pylorus to prevent reflux of duodenal contents into the stomach); a Silastic drainage tube (Dow Corning; i.d., 0.078 in.; o.d., 0.125 in.) through the stomach wall; a Silastic drainage tube (i.d., 0.132 in.; o.d., 0.183 in.) in the terminal ileum (the ileum was ligated distally); a polyethylene tube (PE 50) in the common bile duct above the entrance of the pancreatic ducts in those animals in which bile was diverted to the outside. All tubes were secured with ligatures and brought to the outside through small puncture wounds in the abdominal wall. The animals were allowed to recover from anesthesia (usually overnight) before experiments were begun. They were kept in restraining cages during recovery and during the 8 hr experiments which followed. Immediately after surgery the intestine was flushed out with 15 ml of isosmo-

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lar saline solution composed of sodium chloride and potassium chloride in a molar ratio of 10:1 warmed to 38°. Thereafter the same saline solution was perfused continuously through the intestine at the rate of 2.4 ml/hour with the aid of a perfusion pump until the experiments were begun.

For the experiments, the saline perfusion was interrupted and the intestine was perfused with a micellar solution of bile salt, glyceryl mono-oleate, and oleic acid, the oleic acid containing a trace of 1-^{14}C-oleic acid. The micellar solution was prepared according to Redgrave (7) except that oleic acid and glyceryl mono-oleate were present in a molar ratio of 2:1 (as in glyceryl trioleate) and the solutions were not sonicated or filtered. They became water clear after a short period of stirring and were stable for many days. The bile salt[2] concentration was 20 m*M*, total lipid 18 m*M* (5.5 mg/ml) and phosphate buffer 150 m*M* with respect to sodium. The pH was 6.5. In some of the later experiments phosphate buffer was replaced with sodium chloride solution and the pH was brought up to 6.5 with sodium bicarbonate. This was done to prevent the decrease in lymph flow which was observed to occur when the phosphate-buffered micelles were perfused.

The micellar solution, warmed to 38°, was perfused for 4 hr at a steady rate of 11.4 ml/hr after a preliminary injection of 3–5 ml to fill the intestine and avoid the temporal problems related to intestinal transit. After 4 hr of perfusion the intestine was washed out with 15 ml of warm saline, and saline perfusion was continued at the rate of 2.4 ml/hr for an additional 4 hr. The effluent from the terminal ileum was collected and pooled over the 8-hr experiment. The total lymph output was obtained hourly from the beginning of the micellar infusion. In some animals lymph output was also obtained before micellar infusion. At the end of the 8 hr the animals were killed with an overdose of sodium pentobarbital and the entire small intestine was removed and homogenized in water. All lymph samples, intestinal effluent, and wall homogenates were measured for volume, total lipid concentration, and ^{14}C activity in the lipid. Total lipid was extracted using the method of Blankenhorn and Ahrens (8). ^{14}C activity was measured in the lipid extracts by liquid scintillation (Packard Tricarb liquid scintillation spectrometer Model 314-EX2) at 73% efficiency.

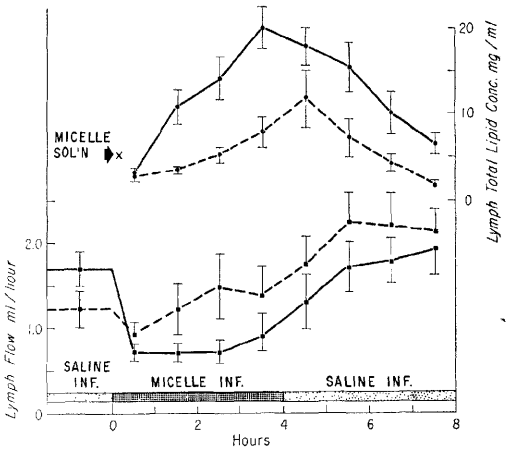

FIG. 1. Lymph flow and lymph total lipid concentration during saline or micelle perfusion. Isosmolar saline (2.4 ml/hr) or micellar lipid solution (11.4 ml/hr) was perfused through the intestine over the periods indicated: Mean of 11 control rats (—); mean of 12 bile fistula rats (- -); vertical bars are standard error of the mean.

Results. Figure 1 shows the effects of micellar perfusion on the lymph flow rate and total lymph lipid concentration during the 8-hr experiments. The lymph lipid represents the sum of the endogenous and absorbed lipids. In the control animals with undisturbed bile flow (Fig. 1, upper) the increase in lymph total lipid concentration during micellar perfusion follows a similar time course initially to that which is seen when triglyceride is injected into the duodenum of normal animals (9). The time to reach peak lipid concentration in this series was highly variable ranging from 3 to 6 hr and this is largely responsible for the high standard errors at each time interval. The peak concentration attained averaged 25.8 mg/ml and ranged from 15.1 to 38.8 mg/ml. Thus in all experiments it exceeded the concentration in

[2] Bile salt was sodium taurocholate, sodium glycocholate, or sodium taurodeoxycholate of the highest purity available (Sigma Chemical Co., St. Louis, Mo.; K and K Laboratories, Inc., Plainview, N. Y.).

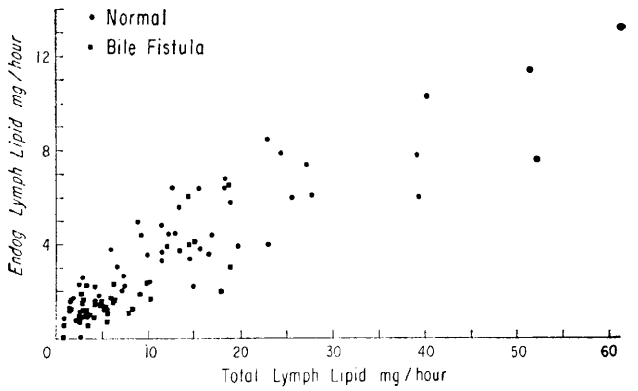

FIG. 2. Endogenous lymph lipid output as a function of total lymph lipid output. ^{14}C-labeled lipid and total lipid were measured in lymph during and following 4 hr of steady intestinal perfusion with ^{14}C-labeled micellar lipid. See text for method of estimating endogenous lipid.

the micellar solution (5.5 mg/ml) by a factor of from 2.7 to 7.0. The lymph samples were milky white throughout most of the time course attesting to the presence of large numbers of chylomicrons.

The rapid increase in lymph lipid of the control animals following micellar perfusion may have been somewhat exaggerated in the present study because of the marked decrease in lymph flow which occurred at the onset of micellar perfusion (see, Fig. 1 bottom). Initially the micellar solutions were made up in phosphate buffer and the phosphate was apparently responsible for the decrease in flow. In later experiments we attempted to avoid this difficulty by making up the micellar solutions in saline and adjusting the pH with sodium bicarbonate. Most of these later experiments, however, were done on bile fistula animals and the higher lymph flows obtained in these animals (see Fig. 1, bottom) are largely a reflection of this.

In the bile fistula rats it is seen that, except for the 5th hour, the total lipid concentration was significantly lower than in the normal group (Fig. 1, upper). As shown below, this was only partly due to the higher lymph flow rates attained in the bile fistula rats. In 5 of the 12 bile fistula rats there was no change in the lymph lipid concentration either during or following the micellar perfusion. The time to achieve peak lipid concentration in those animals which showed an increase was again variable as in the normal group.

There is a possibility that some of the differences in lymph lipid concentration between the normal and bile fistula rats were due to differences in endogenous lymph lipid. The ^{14}C-labeled oleic acid was incorporated into the micellar lipid in order to take this into account. In Fig. 2, endogenous lymph lipid is plotted as a function of the total lipid in the two groups and no apparent differences between the normal and bile fistula rats are evident. Endogenous lipid was calculated from the formula:

$$E = T - \frac{L}{SA_{inf}}$$

where E = endogenous lymph lipid output (mg/hr); T = total lymph lipid output (mg/hr); L = ^{14}C recovered in lymph lipid (counts/hr); SA_{inf} = specific activity of micellar lipid (counts/min/mg).

It was assumed that the labeled oleic acid was absorbed and transported in the lymph at the same rate as the glyceryl mono-oleate and the unlabeled oleic acid. In both groups it is seen that endogenous lipid output increased as the total lipid output increased, suggesting the incorporation of lipid components of endogenous origin into chylomicrons in proportion to their rate of formation.

Perhaps the most significant differences between normal and bile fistula rats are brought out in the data relating to the quantitative distribution and recovery of the ^{14}C-lipid as distinct from the total lipid. These

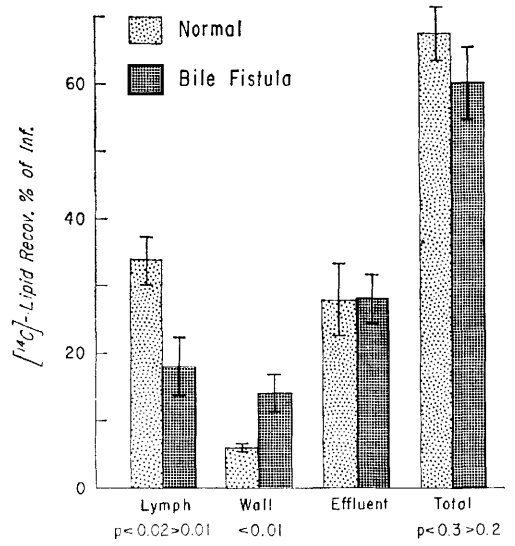

FIG. 3. Cumulative ^{14}C-lipid recoveries in lymph, intestinal wall, and ileal effluent. ^{14}C-lipid was measured 8 hr after steady perfusion of ^{14}C-labeled micellar lipid through the intestine for 4 hr. Vertical bars represent standard error of the mean. p represents probability using the standard t test.

data are presented in Fig. 3. ^{14}C-lipid recoveries in lymph, wall, and ileal effluent, at the end of 8 hr, are given as a percentage of the total amounts infused. In the normal group an average of 33.6% of the label was recovered in the lymph lipid compared to 17.9% in the bile fistula group. The difference is significant at the 2% probability level using the standard t test. Thus the bile fistula animals showed a lower ^{14}C-lipid recovery despite a higher average lymph flow rate. In the wall of the gut the opposite situation pertained, for the bile fistula rats showed a retention of ^{14}C-lipid in the wall which was significantly higher than in the normal group after 8 hr. Thus the reduced lymph recovery in the bile fistula group was due, at least in part, to a lower rate of transport through the wall. It was not due to a reduced absorption rate since the 2 groups showed similar recoveries in the ileal effluents. The total ^{14}C-lipid recovered in all 3 compartments (lymph + wall + effluent) averaged 67.3% in the normal group and 59.8% in the bile fistula group (Fig. 3). This difference is not significant. Thus a sizable portion of the label was absorbed through non-lymph channels.

Discussion. This study, along with recent studies by others (7, 10), supports the validity of the micellar concept. We have shown that chylomicrons are produced from oleic acid and glyceryl mono-oleate perfused through the gut in micellar form. The lipid concentration in the lymph of the normal animals reached as high as 38.8 mg/ml (43.8 m*M* in terms of triolein) and thus exceeded that in the perfusate by as much as sevenfold. It is possible that higher peak lipid concentrations would have been achieved by extending the perfusion period beyond 4 hr. However, Redgrave (7) in 8-hr steady perfusions of micellar lipid at the rate of 3 ml/hr achieved steady state lymph lipid concentrations usually in less than 4 hr. In the present study, the perfusion rate was set at the (perhaps extreme) level of 11.4 ml/hr in order to make as much lipid as possible available to the absorbing surfaces. Even at this high rate 60–70% of the micellar lipid was absorbed or disappeared from the perfusate. Thus the micelles appear to be absorbed at rapid rates which is commensurate with the idea that they must be formed and absorbed at rapid rates normally if all dietary triglyceride goes through a micellar phase. The absorption rate appears not to be the rate-limiting step since the lipid accumulates in the wall and is subsequently transported away at slower rates and in much higher concentrations.

Endogenous lymph lipid increases in proportion to the total lipid during micellar absorption. This agrees with the finding of Redgrave (7) and was perhaps not unexpected since it has been shown that some phospholipid and possibly other lipid components are incorporated into chylomicrons at some point in their production (11, 12). In the present study we found that the increase in endogenous lipid was proportional to the total lipid in both normal and bile fistula rats. Thus we can probably rule out bile as a significant source of the added endogenous lipid even though, as has been suggested (13), some of the endogenous lipid in the lymph of fasting animals may originate in the bile.

There were clear-cut differences between the bile fistula animals and the control animals in terms of the amount of micellar ^{14}C-lipid appearing in the lymph and the amount retained in the gut wall after 8 hr, although both groups absorbed similar amounts. The bile fistula group retained more of the label in the wall after 8 hr than did the normal group and, probably partly as a result of this, there was less recovered in the lymph. The bile salt concentration in the perfusates was 20 m*M*, well above the critical micellar concentration (3). Since the effluents collected from the terminal ileum remained clear in both groups of animals it may be assumed that the lipid remained in micellar form during passage through the gut even in the absence of natural bile.

Some previous studies (4, 5) have shown that the diversion of bile from the intestine reduces the absorption of lipid while allowing for a larger than normal fraction of that which is absorbed to appear in the portal blood as unesterified fatty acid. As a consequence it has been assumed that the bile salts play a role in facilitating fatty acid esterification in the mucosa (5). In the present study, we supplied bile salts in concentrations well above the critical micellar concentration in both groups of animals but still failed to achieve normal rates of lymph lipid transport in the bile fistual group. This suggests the possible existence of a non-bile salt component in natural bile which plays a role in the phase of lipid absorption involved with transport through the wall or lymph. However, it is possible that other disturbances of unknown nature result when the enterohepatic recirculation is interrupted. Whatever the mechanism, in terms of micellar lipid transport the bile salts which we used in the preparation of the micelles did not adequately fulfill the functions of natural bile despite their presence in high concentration.

Summary. The small intestines of control and bile fistula rats were perfused for 4 hr with a micellar solution of bile salt, glyceryl monooleate, and ^{14}C-labeled oleic acid followed by rapid washout and steady perfusion with saline for an additional 4 hr. Measurements on hourly lymph collections from the thoracic duct for 8 hr, pooled ileal effluents and intestinal wall showed that (i) in both groups endogenous lymph lipid increased in proportion to the total lipid, (ii) in the control group lymph lipid increased after 3–6 hr to peak concentrations of from 15.1 to 38.8 mg/ml or 2.7–7.0 times that of the micellar solution, (iii) in both groups similar amounts of the micellar lipid were absorbed but the bile fistula group retained a significantly higher proportion in the wall after 8 hr and transported a significantly lower proportion in the lymph. The results support the micellar concept of lipid absorption by showing the production of chylomicrons from micelles in control animals but indicate that chylomicron production or transport is abnormal in bile fistula animals despite the presence of bile salts in the lumen above the critical micellar concentration.

1. Verzar, F., in "Absorption from the Intestine," p. 160. Longmans Green and Co. (1936).

2. Hofmann, A. F., Gastroenterology **50**, 56 (1966).

3. Hofmann, A. F., Nature (London) **190**, 1106 (1961).

4. Borgström, B., Acta Physiol. Scand. **28**, 279 (1953).

5. Saunders, D. R., and Dawson, A. M., Gut **4**, 254 (1963).

6. Bollman, J. L., Cain, J. C., and Grindlay, J. H., J. Lab. Clin. Med. **33**, 1349 (1948).

7. Redgrave, T. G., Quart. J Exp. Physiol. **52**, 130 (1967).

8. Blankenhorn, D. H., and Aherns, E. H., Jr., J. Biol. Chem. **212**, 69 (1955).

9. Rampone, A. J., Proc. Soc. Exp. Biol. Med. **108**, 278 (1961).

10. Hofmann, A. F., and Borgström, B., J. Lab. Clin. Med. **43**, 247 (1964).

11. Rampone, A. J., Amer. J. Physiol. **199**, 1015 (1960).

12. Bollman, J. L., Flock, E. V., Cain, J. C., and Grindlay, J. A., Amer. J. Physiol. **163**, 41 (1950).

13. Shrivastava, B. K., Redgrave, T. G., and Simmonds, W. J., Quart. J. Exp. Physiol. **52**, 305 (1967).

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