PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 166, 12–16 (1981)

# Dietary Fibers

## VI: Binding of Fatty Acids and Monolein from Mixed Micelles Containing Bile Salts and Lecithin (41016)[1]

G. V. VAHOUNY, R. TOMBES, M. M. CASSIDY, D. KRITCHEVSKY, AND L. L. GALLO

*Department of Biochemistry, The George Washington University School of Medicine and Health Sciences, Washington, D.C. 20037 and The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104*

*Abstract.* Mixed micelles were prepared containing sodium taurocholate, monolein dioleyl lecithin, cholesterol, and an equimolar mixture of palmitic, oleic, and linoleic acids. These were incubated with commercial bile acid-sequestering resins, cholestyramine and DEAE-Sephadex, or various dietary fibers and fiber components including wheat bran, cellulose, alfalfa, lignin, and two viscosity grades of guar gum. Binding of monolein and fatty acids was determined as the difference between the radioactivity of the added micellar component, and that recovered in the centrifugal supernatant after incubation. In general, the extent of monoglyceride or fatty acid sequestration was characteristic and reproducible for each binding agent. Cholestyramine and DEAE-Sephadex essentially quantitatively bound monoglycerides and all three fatty acids from micellar medium. Low- and high-viscosity grades of guar gum sequestered 15–23% of the monolein and 32–33% of the fatty acids, showing a significant preference for linoleic acid in each case. Alfalfa fiber removed about 18% of the micellar monoglyceride and mixed fatty acids, again showing some preference for the polyunsaturated acid. Lignin, the hydrophobic component of dietary fibers, sequestered about 13% of the available lipids and displayed an apparent preference for oleic acid. Wheat bran and cellulose showed little affinity for micellar lipids binding about 11 and 4.7%, respectively. These data on resin and fiber sequestration of micellar fatty acids and monoglycerides compare favorably with the binding of other micellar components including phospholipid, bile salt, and cholesterol.

It has been suggested that increased intake of certain dietary fibers or fiber isolates may have hypocholesteremic properties in humans (1, 2) and experimental animals (3, 4). These include fibers which tend to sequester bile acids *in vitro* (5, 6), and may exhibit hypocholesteremic properties by increasing excretion of these acidic steroids *in vivo* (e.g., (4)). For example, alfalfa fiber has been reported to be hypocholesteremic (3), to increase fecal excretion of bile acids (7) and to bind these substances *in vitro* (5, 6). Generally, the efficacy of dietary fibers in sequestering bile acids has been compared to commercial ion-exchange resins used therapeutically to reduce hypercholesteremia on the basis of their bile acid sequestration properties (8, 9).

We have recently reported (6) that "bile acid-sequestering" resins are also effective sequestrants of other components of mixed micelles typically present in the intestinal lumen. These include the phospholipid, lecithin, and cholesterol, in addition to the bile salts, sodium taurocholate, and taurochenodeoxycholate. The sequestration of biliary lecithin, and subsequent interference with reabsorption and utilization in synthesis of lipoprotein were suggested to account for the accumulation of intestinal cell lipids observed when these sequestrants were fed chronically.[2]

In the present study we have investigated the *in vitro* ability of commercial ion-

[1] Supported in part by USPHS Grants HL-02033 (G. V. V.) and HL-02399 (D. K.), Research Career Award HL-0734 (D. K.) and a grant from the United States Department of Agriculture 7900497 (G. V. V.).

[2] M. M. Cassidy and Vahouny G. V., unpublished observations.



0037-9727/81/010012-05$01.00/0

exchange resins and fibers to adsorb or sequester monoglycerides and fatty acids, which physiologically results from fat digestion and are typical components of intraluminal mixed micelles. Binding has been determined using mixed micelles containing bile salts, lecithin, cholesterol monoglyceride, and fatty acids. Because of the nutritional interest in saturated and unsaturated fats, fatty acids have been included as an equimolar mixture of palmitic, oleic, and linoleic acids.

**Materials and Methods.** Monolein, fatty acids (<99% purity), dioleyl lecithin, and sodium taurocholate were obtained from Sigma Chemical Company, St. Louis, Missouri. Cholesterol was purchased from Supelco, Bellefonte, Pennsylvania. Labeled compounds were obtained from New England Nuclear Corporation, Boston Massachusetts, and Amersham-Searle, Arlington Heights, Illinois.

Mixed micelles were prepared as previously described (10) and contained each component within physiological ranges. These included 5 m*M* sodium taurocholate, 625 $\mu M$ lecithin, 250 $\mu M$ cholesterol, 250 $\mu M$ monolein, and 500 $\mu M$ of an equimolar mixture of palmitic, oleic and linoleic acids, and contained various combinations of mono-[1-$^{14}$C]olein, [1-$^{14}$C]palmitic acid, [1-$^{14}$C]-linoleic acid, and [9,10-$^{3}$H]oleic acid for analysis of binding by liquid scintillation spectrometry.

Micellar media were prepared by mixing the lipids in hexane with the bile salt in physiological saline. After evaporation of the solvent at 25°C under nitrogen, complete solution in physiological saline was achieved by mechanical agitation for 30–60 sec. The solutions were then centrifuged at 105,000*g* for 1 hr and the clear infranatant, representing micellar bile salts and lipid, was analyzed by liquid scintillation spectrometry.

Binding of lipids were determined as previously described for bile acids (11, 12) and employed earlier in this laboratory (13, 14). The test materials included cholestyramine (Questran; Dr. H. P. Sarett, Mead Johnson, Evansville, Ind.); DEAE-Sephadex (Secholex, a gift from Dr. A. Howard, Cambridge, England); cellulose (Solka flok, Brown and Co., Berlin, NH); alfalfa and white wheat bran (Bio-Serv, Frenchtown, N.J.); lignin (Westvaco, Charleston, S.C.); and two viscosity grades of guar gum (very high viscosity, Hathway; low viscosity, food grade, Hercules, Inc., Wilmington, Del.). Forty milligrams of the ion-exchange resin or dietary fiber was added to 5 ml of each micellar solution in a stoppered tube and the mixture was shaken at 37° for 1 hr. The tubes were centrifuged at 30,000*g* for 10 min and the entire supernatant was unified by homogenization prior to assay. Aliquots (0.1 ml) of the supernatant were added to 10 ml liquid scintillation (Scintiverse, Yorktown Research, Elmhurst, Pa.), and radioactivity was determined in a Beckman LS 250 liquid scintillation spectrometer using external standardization. Controls of the appropriate micellar media without added binding substances were carried through the same procedure. Binding was determined as the difference between the radioactivity of each micellar component added and that recovered in the supernatant after incubation. All studies were carried out in triplicate and all isotope analyses were carried out in duplicate.

**Results and Discussion.** The conditions for determining bile acid and phospholipid sequestration from micellar solutions had previously been established using cholestyramine as the test material (6). The specific binding of bile salts from micellar media might result in dissociation of the micelles, resulting in reduced solubility of other micellar lipids. This was tested by assaying for labeled micellar components along the length of the tube following incubation with the resin and centrifugation. Under these conditions "unbound" lipids were found to float. Thus in all studies, the supernatants from the 30,000*g* centrifugation were unified by homogenization prior to isotope analyses. These earlier studies also investigated reequilibration of "prebound" bile salts and phospholipids, when reincubated in unlabeled micellar media. The results suggested that a consistent and reproducible amount of bile salt, phospholipid, and cholesterol were sequestered by the ion-exchange resins and that this was not readily released into aqueous micellar

TABLE I. BINDING OF INDIVIDUAL MICELLAR COMPONENTS BY CHOLESTYRAMINE

| Labeled components[a] | Percentage binding | | | | |
|---|---|---|---|---|---|
| | Bile salt | Lecithin | Chol. | Oleic acid | Monolein |
| [$^3$H]Taurocholate[b] | 78.0 ± 0.5 | — | — | — | — |
| Taurocholate + [$^{14}$C]lecithin | 79.3 ± 0.7 | 91.0 ± 0.7 | — | — | — |
| Taurocholate + [4-$^{14}$C]cholesterol | 75.2 ± 1.1 | — | 96.2 ± 0.4 | — | — |
| Taurocholate + [1-$^{14}$C]oleic | 79.3 ± 0.7 | — | — | 95.7 ± 0.6 | — |
| Lecithin + [4-$^{14}$C]cholesterol | — | 92.1 ± 1.0 | 92.4 ± 0.9 | — | — |
| Taurocholate + mono[1-$^{14}$C]olein | 77.6 ± 0.3 | — | — | — | 96.3 ± 1.1 |

[a] All micellar preparations contained 5 m*M* taurocholate, 625 $\mu M$ lecithin, 250 $\mu M$ monolein, 500 $\mu M$ oleic acid, and 250 $\mu M$ cholesterol. Triplicate incubations were carried out with 40 mg cholestyramine and contained the indicated combinations of labeled substances in the micellar mixture. Figures represent means from six determinations ± SEM.

[b] [n-$^3$H]taurocholic acid.

media. The results in Table I summarize similar studies on cholestyramine sequestration of various components of mixed micelles containing bile salt and lecithin as major amphipaths, monolein and oleic acid as amphiphiles, and cholesterol. These represent the major components in intestinal contents under physiological conditions. In each case, two of the five micellar components were labeled with $^{14}$C and $^3$H to allow dual label liquid scintillation spectrometry, and to obtain appropriate overlap of binding data. As has been shown in earlier studies using bile salts alone (15) or in mixed micelles (6), cholestyramine sequestration of sodium taurocholate is reproducibly incomplete ranging from 75 to 79% of that present. These studies also confirm those reported by us earlier concerning the extensive resin sequestration of lecithin and cholesterol from micellar media (6). In addition, it is also apparent that the binding of the two amphiphiles, oleic acid and monolein, is essentially quantitative.

Fatty acid and monolein binding by ion-exchange resins and dietary fibers were conducted using micellar preparations containing equimolar mixtures of palmitic (18:0) oleic (18:1 $\omega$9) and linoleic (18:2 $\omega$6) acids, in order to determine whether fatty acid chain length or unsaturation was a determinant in sequestration. Each of the identical micellar preparations also contained various combinations of two labels includ-

TABLE II. BINDING OF FATTY ACIDS AND MONOLEIN FROM BILE SALT PHOSPHOLIPID MICELLES TO ION-EXCHANGE RESINS AND DIETARY FIBERS

| Test substance[a] | Percentage binding[b] | | | | |
|---|---|---|---|---|---|
| | Palmitic | Oleic | Linoleic | All fatty acids | Monolein |
| Cholestyramine | 96.6 ± 0.6 | 92.6 ± 0.9 | 97.9 ± 0.2 | 95.7 ± 0.6 | 96.3 ± 1.1 |
| DEAE-Sephadex | 99.2 ± 0.2 | 94.9 ± 0.2 | 99.8 ± 0.02 | 97.9 ± 0.3 | 99.3 ± 0.2 |
| Guar gum, L.V.[c] | 31.3 ± 1.4 | 31.4 ± 2.1 | 37.0 ± 0.9 | 33.2 ± 1.5 | 23.1 ± 2.8 |
| Guar gum, H.V.[d] | 29.2 ± 3.8 | 25.1 ± 2.2 | 40.2 ± 0.9 | 31.5 ± 2.3 | 14.6 ± 1.6 |
| Alfalfa | 14.8 ± 0.9 | 15.2 ± 0.8 | 23.8 ± 0.5 | 17.9 ± 0.9 | 18.6 ± 1.4 |
| Lignin | 10.2 ± 0.7 | 19.5 ± 0.9 | 6.4 ± 0.3 | 13.0 ± 0.6 | 12.8 ± 1.0 |
| Bran | 9.6 ± 1.1 | 10.4 ± 1.1 | 13.0 ± 1.0 | 11.6 ± 1.1 | 11.3 ± 0.8 |
| Cellulose | 2.3 ± 0.7 | 5.8 ± 1.0 | 3.6 ± 0.6 | 3.9 ± 0.8 | 3.5 ± 1.0 |

[a] Micellar mixture contained 5 m*M* taurocholate, 625 $\mu M$ lecithin, 250 $\mu M$ monolein, 500 $\mu M$ fatty acid, and 250 $\mu M$ cholesterol. Triplicate incubations were carried out with 40 mg of each test substance and contained various combinations of [1-$^{14}$C]palmitic acid, [1-$^{14}$C]linoleic acid, [9,10-$^3$H]oleic acid, and mono[1-$^{14}$C]olein.

[b] Figures represent means from 6–12 incubations ± SEM.

[c] Guar gum, low-viscosity food grade.

[d] Guar gum, high viscosity.

ing mono[1-$^{14}$C]olein, [9,10-$^{3}$H]oleic acid, [1-$^{14}$C]linoleic acid, and [1-$^{14}$C]palmitic acid.

The data, summarized in Table II, shows that both commercial ion-exchange resins, cholestyramine and DEAE-Sephadex (Secholex) sequester fatty acids and monolein from micellar mixtures quantitatively, and that there is no distinction for fatty acid chain length or unsaturation. The two preparations of guar gum gave comparable results, binding 32–33% of the mixed fatty acids and 15–23% of the monolein. These fibers were previously shown to sequester 38–36% of the bile salt, 22–38% of the phospholipid, and 18–23% of the cholesterol from micellar preparations (6). Because of the difficulty in studying binding to these viscous fibers, as attested to by the higher statistical variations, differences in monolein binding by the two viscosity grades of guar gum were not considered as significant. It was of interest, however, that both preparations appeared to sequester linoleic acid to a greater extent and with a smaller variance in results.

Alfalfa which has been reported to have hypocholesteremic properties (3) and causes increased excretion of fecal acidic steroids (7), sequestered about 18% of the fatty acids and monolein from micelles and, as in the case of guar gum, appeared to prefer linoleic acid in the equimolar fatty acid mixture. The fiber, however, showed little affinity toward the phospholipids or cholesterol in micelles (6).

Lignin, a hydrophobic component of dietary fibers has been reported to have bile acid-sequestering properties (16) but is less efficient in "binding" phospholipids or cholesterol (6). This fiber isolate sequestered about 14% of the available monolein and fatty acids and displayed an apparent preference for oleic acid.

Wheat bran and cellulose have previously been shown to have poor bile acid-sequestering properties (5, 15) and show little affinity for phospholipids or cholesterol (6). In the present study, monolein or fatty acid binding by wheat bran and cellulose were about 11 and 4%, respectively, with little indication of preference for fatty acid chain length or unsaturation.

The present studies, together with those presented earlier (6, 13), suggest that commercial bile acid sequestrants and certain dietary fibers or fiber isolates, such as the viscous and hydrophobic fibers, are relatively unselective with respect to "sequestration" of individual components of mixed micelles *in vitro*. A similar affinity for these intraluminal molecular aggregates *in vivo* would not only reduce the availability of bile salts for active reabsorption in the lower ileum but also result in decreased availability of cholesterol and the products of triglyceride digestion for absorption in the upper jejunum. In addition, sequestration of biliary lecithin might interfere with subsequent availability of lysolecithin for reabsorption and subsequent utilization as lecithin in chylomicron and very-low-density lipoprotein formation in the intestinal epithelium. Any one or more of these properties could explain the high correlation between altered lymphatic absorption of both cholesterol and triglycerides in rats fed cholestyramine and various dietary fibers (14), and the morphological evidence of lipid accumulation in intestinal epithelium of rats fed 2% levels of "bile acid"-binding resin for 4–6 weeks.[2]

1. Keys, A., Grande, F., and Anderson, J. T., Proc. Soc. Exp. Biol. Med. **106**, 555 (1961).
2. Jenkins, D. J. A., Leeds, A. R., Slavin, B., Mann J. and Jepsom, E. M., Amer. J. Clin. Nutr. **32**, 16 (1979).
3. Cookson, F. M., and Federoff, S., Brit. J. Exp. Pathol. **49**, 348 (1968).
4. Leveille, G. A., and Sauberlich, H. E., J. Nutr. **88**, 207 (1966).
5. Kritchevsky, D. and Story, J., J. Nutr. **104**, 458 (1974).
6. Vahouny, G. V., Tombes, R., Cassidy, M. M., Kritchevsky, D., and Gallo, L. L., Fed. Proc. **39**, 715 (1980).
7. Reddy, B. S., Watanabe, K., and Sheinfil, A., J. Nutr. **110**, 1247 (1980).
8. Huff, J. W., Gillfillan, J. L. and Hunt, V. M., Proc. Soc. Exp. Biol. Med. **114**, 252 (1963).
9. Grundy, S. M., Ahrens, E. H., and Salen, G., J. Lab. Clin. Med. **78**, 94 (1971).

10. Thornton, A. G., Vahouny, G. V., and Treadwell, C. R., Proc. Soc. Exp. Biol. Med. **127**, 629 (1968).
11. Hagerman, L. M., Julow, D. A., and Schneider, D. R., Proc. Soc. Biol. Med. **43**, 89 (1973).
12. Kritchevsky, D., and Story, J. A., Amer. J. Clin. Nutr. **28**, 805 (1975).
13. Vahouny, G. V., Roy, T., Gallo, L. L., Story, J. A., Kritchevsky, D., Cassidy, M., Grund, B. M., and Treadwell, C. R., Amer. J. Clin. Nutr. **31**, 208 (1978).
14. Vahouny, G. V., Roy, T., Gallo, L. L., Story, J., Kritchevsky, D., and Cassidy, M. M. Amer. J. Clin Nutr. **33**, 2182 (1980).
15. Story, J. A., and Kritchevsky, D., J. Nutr. **106**, 1292 (1976).
16. Eastwood, M. A., and Mowbray, L., Amer. J. Clin. Nutr. **29**, 1461 (1976).

---

Received May 7, 1980. P.S.E.B.M. 1981, Vol. 166.