Correlation between Bile Secretion and Triglyceride Release in Perfused Rat Liver and Some Effects of Fenfluramine (36231)

JULIAN B. MARSH, AMALIA GUAITANI, IVAN BARTOSEK, AND ADALGISA BIZZI
Istituto di Ricerche Farmacologiche Mario Negri, Via Eritrea 62, 20157, Milan, Italy

During experiments designed to study the effects of fenfluramine on triglyceride secretion by rat liver, we carried out a number of liver perfusions. When the rate of bile secretion was compared with the release of triglycerides into the medium, a significant correlation was noted. To our knowledge, such an observation had not been reported previously.

Methods. Livers of male albino rats (180–220 g, fed *ad libitum* on laboratory chow) were isolated and perfused (without heparin) as previously described (1), except that before attachment to the apparatus, the livers were perfused with Krebs–Ringer–bicarbonate solution (saturated with 5% CO_2–95% O_2) to remove as much blood as possible. The perfusion medium consisted of 50 ml of a suspension of washed rat erythrocytes (hematocrit, 0.15) in specially prepared rat serum diluted with an equal volume of Krebs–Ringer–bicarbonate, pH 7.4. The rat serum was previously centrifuged for 16 hr at 4° in a Spinco 40 rotor. The top 10% of the volume of each tube was removed, thereby depleting the serum of chylomicrons and very low density lipoproteins. The perfusion rate was 1 ml/g of liver/min. Samples of medium were removed after 5 min (initial sample), 65 min, and 125 min, at which time the perfusion was stopped; and the liver was weighed. After centrifugation of the samples of medium, the supernatant fluid was analyzed for triglyceride content (2); and the net output per gram of final wet weight of liver was calculated.

Results. The relationship between bile secretion and triglyceride release in 21 perfusions is shown in Fig. 1. The regression equation for the straight line is: triglyceride output $= 0.741 + 16.8$ (bile flow-0.0452) and the correlation coefficient is 0.73. During the second hour, bile flow was 78 $\pm$ 5.2% of that measured in the first hour; triglyceride output was 52 $\pm$ 4.0%. However, a significant correlation between bile flow and triglyceride output was also obtained with the 1 or 2 hr rates considered separately.

There have been many studies of triglyceride secretion by the isolated perfused rat liver since the original work of Kay and Entenman (3), but no systematic correlations have been made with any functional parameters of the liver during the perfusion, such as bile flow or oxygen uptake. Hems *et al.* (4), in their study of gluconeogenesis, found large increases in oxygen uptake with added lactate. Their values for bile flow were 0.067 g/g of liver/hr. Our rates are not strictly comparable because of differences in perfusion medium and the use of a constant flow apparatus, but they are in agreement with other values reported, such as 0.033 g/g/hr found by Percy-Robb and Boyd (5).

Because of the interest in this laboratory in the effects of fenfluramine, a drug which lowers plasma triglyceride levels in rats (unpublished data), we perfused livers from rats injected intraperitoneally with 20 mg/kg of *dl*-fenfluramine. At 2 or 4 hr after injection, the livers were removed and perfused. According to Fig. 1, the relationship between bile flow and triglyceride release is the same as in the controls; and, in fact, the mean triglyceride output was not significantly different from controls. However, in three experiments, the direct addition of fenfluramine to the perfused medium at a concentration of 10^{-4} *M* significantly depressed triglyceride output as well as the rate of bile flow (Fig. 1). Further experiments are needed to determine whether a dose–response relationship

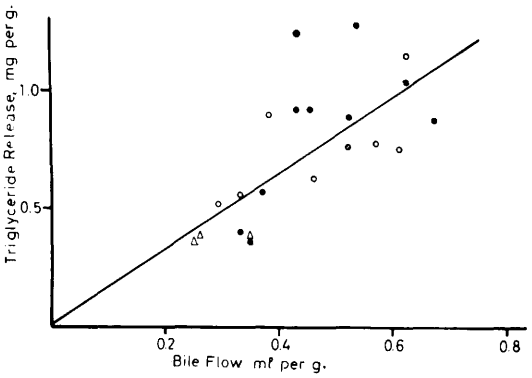

FIG. 1. Relationship between triglyceride release and bile flow in the perfused liver: (●) control rats; (○) rats injected *in vivo* with fenfluramine; (△) normal livers with fenfluramine added *in vitro*.

exists.

The significance of the correlation between bile flow and triglyceride secretion is not clear at present. One possibility is that each liver may show varying degrees of resistance to flow, and that vasoconstriction may result in cellular hypoxia, with a consequent diminution in all metabolic functions. However, fenfluramine *in vivo* produces very little rise in arterial blood pressure (6). When fenfluramine was added to the perfusion medium there was no change in pressure (at constant flow) from the usual value of 70–130 mm H_2O. Furthermore, direct injection of fenfluramine into the portal vein at doses up to 100 μg did not increase the pressure; yet in these experiments there was a marked diminution of bile flow. Control injections of 1 μg of norepinephrine caused an immediate 2–3-fold rise in perfusion pressure.

Klevay and Hegsted (7) reported that rats fed Purina chow secreted a significantly greater volume of bile during perfusion than did a similar group of rats fed purified diets containing safflower or coconut oil. It seems likely, therefore, that individual differences in dietary or hormonal state in each animal may be responsible for the concomitant changes in bile flow and very low density lipoprotein secretion, which is the form in which triglycerides are released by the liver (2).

Summary. A significant correlation has been found between bile flow and triglyceride release by the isolated perfused liver.

This investigation was partially supported by Programma Speciale Tecnologie Biomediche, C. N. R. Contract No. 70 00716/30.17.9.3.

1. Kvetina, J., and Guaitani, A., Pharmacology **2**, 65 (1969).

2. Van Handel, E., Zilversmit, D. B., and Bowmann, K., J. Lab. Clin. Med. **50**, 152 (1957).

3. Kay, R. E., and Entenman, C. J., Biol. Chem. **236**, 1006 (1961).

4. Hems, R., Ross, B. D., Berry, M. N., and Krebs, H. A., Biochem. J. **101**, 284 (1966).

5. Percy-Robb, I. W., and Boyd, G. S., Biochem. J. **118**, 519 (1970).

6. Bizzi, A., Bonaccorsi, A., Jespersen, S., Jori, A., and Garattini, S., *in* "Amphetamines and Related Compounds" (E. Costa and S. Garattini, eds.), p. 577. Raven Press, New York (1970).

7. Klevay, L. M., and Hegsted, D. M., J. Atheroscler. Res. **8**, 329 (1968).

Received Sept. 23, 1971. P.S.E.B.M., 1972, Vol. 139.