Plasma Protein Escape from the Intestinal Circulation to the Lymphatics During Fat Absorption[1] (37188)

A. Wollin and L. B. Jaques

Hemostasis–Thrombosis Research Unit, Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, S7N 0W0

The plasma proteins are known to leave the circulation in small quantities under normal conditions. The rate of exudation varies from organ to organ (1) and is also dependent on factors which increase capillary permeability (2). Excessive plasma protein escape can be readily observed by the aid of a dye label. Evans blue, as used in the following experiments, combines firmly with plasma albumin when injected into the circulation and is thus carried along with the plasma albumin. Any significant escape of this blue-colored complex from the circulation into the connective tissue results in a blue coloration of this particular area and indicates altered vascular permeability (3).

This communication reports a local condition in which there is considerable loss of plasma protein from the circulation as a part of a physiological process. During experiments in which Evans blue was injected intravenously into rats receiving olive oil by stomach tube, it was observed that the duodenum and jejunum assumed a strong blue coloration compared to other tissues. This phenomenon was therefore investigated.

Materials and Methods. Male Wistar rats were used with body weights ranging from 260–360 g receiving the care as outlined by the Guiding Principles enunciated by the Canadian Council on Animal Care.

Visual observation of Evans blue escape. Rats starved overnight were lightly anesthetized with ether and tube fed olive oil (2 ml/kg body weight) or an equivalent volume of glucose solution (0.5 g/ml) as control. Evans blue (10 mg/kg body weight, 0.5% solution, Warner-Chilcott, Morris Plains, New Jersey) was injected into the lateral tail vein. After 1 hr the rats were deeply anesthetized with ether and the tissues of the abdominal cavity and the skin visually examined for the blue coloration.

Determination of the disappearance rate of Evans blue from circulation. Male rats were lightly anesthetized with ether. Each rat was placed in a restraining cage and Evans blue (10 mg/kg body weight) injected intravenously. Blood samples were taken from a cut at the tip of the tail at approximately 5-min intervals. A volume of 0.1 ml of blood was milked into the well of a porcelain plate, containing a small amount of dry heparin. The blood was mixed and 0.05 ml removed for the determination of the Evans blue concentration by a method as described by Savoie (4). This technique permitted the removal of a considerable number of small blood samples without seriously affecting the total blood volume. The experiment was done with three starved and three olive oil fed rats.

Lymph collection and analysis. The large intestinal lymph vessel was cannulated as described by Bollman *et al.* (5). Following the surgery each rat was kept in a restraining cage overnight with free access to 0.5% NaCl solution to allow recovery and stabilization from the surgical procedure. Lymph samples were collected in an 8-ml vial, containing some heparin to prevent coagulation. During the sample collection the rat was conscious, had free access to 0.5% NaCl solution and was shaded from light by a cover.

Evans blue (10 mg/kg body weight) was first injected into the lateral tail vein under

[1] This research was supported by a grant-in-aid to Dr. L. B. Jaques from the M. R. C. of Canada (Grant No. MA 2744).

light ether anesthesia, followed by intubation of olive oil (2 ml/kg body weight) or an equivalent volume of a glucose solution (0.5 g/ml). Lymph was collected over eight one-hour periods starting 10 min after injection of the Evans blue. The volume of each hourly collection was measured and the samples stored under refrigeration for the determination of Evans blue concentration (4). The protein concentration was estimated by electrophoresis on Sepraphore III, a cellulose polyacetate supporting media (Gelman Instrument Co.), for a series of lymph samples with varying Evans blue concentrations. The collection procedure was carried out on five olive oil fed rats and six rats fed glucose solution.

Results. When rats fed olive oil following deprivation of food overnight were injected with Evans blue, a marked blue coloration of the small intestines was observed. This strong coloration appeared only in the duodenum, jejunum and in the lymph nodes of the adjacent mesentery and to some extent in the ileum and snout. The rats receiving an equivalent volume of glucose solution of olive oil did not show the marked blue coloration. Their small intestines showed only light blueing. The other tissues showed only a trace of blueing in both olive oil-fed and glucose-fed animals. Rats fed pellets containing the usual laboratory mixed diet and injected with Evans blue, showed blueing of the small intestines intermediate between that seen with glucose and that seen with olive oil.

Several questions arose from the above experiments. Had the dye complex escaped from the circulation or was the blue color due to an increased blood supply and proportional increase in Evans blue present? Since a relatively large amount of dye was given and since fatty acids entering the blood during olive oil feeding are transported by albumin and could displace the Evans blue from the plasma albumin, it was questioned whether the blue coloration had resulted from the escape of free Evans blue into the interstitial space or the dye complex. Hence the rate of loss of Evans blue from the circulation was examined. The results are shown in Fig. 1 in which values for

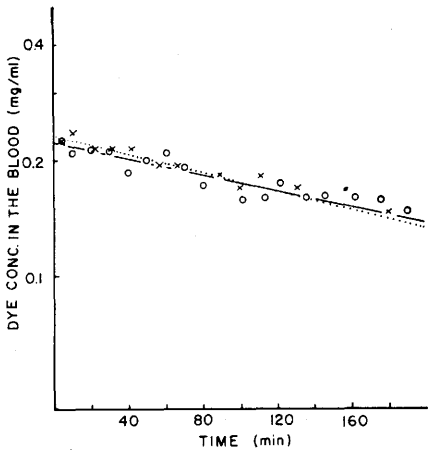

FIG. 1. The loss of Evans blue from the rat blood after a single injection of Evans blue. Regression line calculated for each rat. X: rats fed glucose, O: rats fed olive oil.

the log of Evans blue concentration in the blood versus time were plotted. The loss of dye from the circulation showed the same linear relationship for rats with and without olive oil feeding prior to the Evans blue injection, since the calculated regression lines were almost identical. The slow disappearance of Evans blue from the circulation was similar to the response reported by Gregersen and Rawson (6) with a much lower dye concentration. This data gives evidence that at the Evans blue concentration used, the dye was mostly bound to plasma albumin and not free.

In order to quantitate changes in the protein loss from the intestinal circulation, the relation between Evans blue concentration and plasma protein concentration in the lymph was examined. Values obtained for the lymph protein concentration and lymph albumin concentration were plotted against the corresponding Evans blue concentration. The results are reported in Fig. 2. This plot gave a linear relationship for both total protein and albumin, and demonstrates that Evans blue concentration in the lymph reflects the escaped Evans blue–protein complex from the circulation.

The quantitative effect on escape of plasma protein from the intestinal circulation of feeding olive oil was examined in rats with a lymph fistula. Lymph collected for each

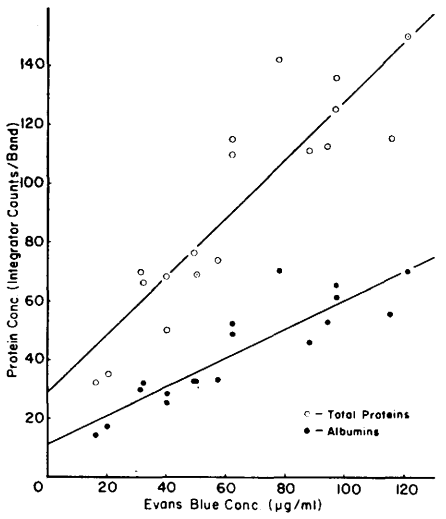

FIG. 2. The relationship between Evans blue concentration and plasma protein concentration in rat intestinal lymph.

hour, was measured and the concentration of Evans blue determined. The total amounts of Evans blue collected at each one hour period are shown in Fig. 3. Rats fed olive oil showed a considerable increase in Evans blue in the lymph compared to similarly prepared rats receiving glucose solution. During the period from two to three hours after olive oil intubation, the rate of Evans blue collection was at least three times the amount collected while feeding glucose during the same time period. The increase in amounts of Evans blue collected was due to a combination of an increased lymph flow and an increase in Evans blue concentration in lymph.

Discussion. Only a light blue coloration is seen in the tissues of starved rats when injected intravenously with Evans blue. An intense blueing of any tissue is an indication of Evans blue rapidly leaving the circulation, along with albumin to which it is bound, and accumulating in the interstitial space (2). On the basis of the experiments reported in Figs. 1 and 2, we concluded that the Evans blue–protein concentration in the lymph is indicative of the escape of plasma proteins from the circulation. The corresponding data shown in Fig. 3 suggests that olive oil absorption causes a local vascular change, allowing an increased escape of plasma proteins. Whether this effect is the result of an increased capillary permeability, a shunting of the blood flow to an exchange capillary network (7) or endothelial vesicular transport is not clear. The intestinal capillaries have a greater permeability than the peripheral capillaries (1) and therefore a greater transcapillary movement of the Evans blue–albumin complex would be expected if the capillary flow is increased on

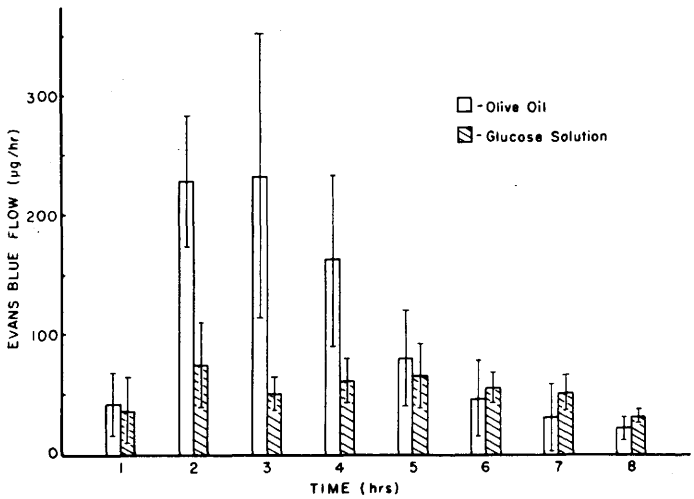

FIG. 3. The effect of olive oil and glucose feeding on the escape of Evans blue from the circulation in the rat intestines. Olive oil: 5 rats; Glucose solution (control): 6 rats.

account of metabolic requirements. However, an increase in capillary flow during olive oil absorption alone could not completely explain this observation, because glucose feeding did not increase the protein escape. During glucose feeding an increase in blood flow would be expected and should cause a similar rise in the quantity of protein in lymph fluid. This did not occur (Fig. 3).

Previous studies of the effect of feeding of various nutrients on lymph have been limited to thoracic duct lymph. Simmonds (8, 9) found olive oil and oleic acid increased lymph flow without a decrease in protein level in the lymph sample and Gallo-Torres and Miller (10) found only the triglycerides increased lymph flow in the absence of saline. Since the thoracic duct drains a number of organs, the source of the lymph changes observed were not defined by these workers.

Summary. An escape of plasma proteins labeled with Evans blue was observed in the small intestines of rats. Lymph was collected from an intestinal fistula and the rate of protein escape measured by the appearance of Evans blue. During feeding of olive oil the amount of Evans blue collected was much higher than when rats received glucose solution.

We wish to thank Dr. S. S. Naidoo for interest and advice and Mrs. Hoo Ja Oh, Miss Ranju Bakshi, and Mr. Renato Mag-atas for expert technical assistance.

1. Mayerson, H. S., Wolfrom, C. G., Shirley, H. H., and Wasserman, K., Amer. J. Physiol. **198,** 155 (1960).

2. Spector, G. W., Pharmacol. Rev. **10,** 475 (1958).

3. Parratt, J. R., and West, G. B., J. Physiol. **140,** 105 (1958).

4. Savoie, L. L., Can. J. Physiol. Pharmacol. **47,** 241 (1969).

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

6. Gregersen, M. I., and Rawson, R. A., Amer. J. Physiol. **138,** 698 (1943).

7. Chien, S., Circ. Res. **29,** 173 (1971).

8. Simmonds, W. J., Austral. J. Exp. Biol. **32,** 285 (1954).

9. Simmonds, W. J., Austral. J. Exp. Biol. **33,** 305 (1955).

10. Gallo-Torres, H. E., and Miller, O. N., Proc. Soc. Exp. Biol. Med. **132,** 1 (1969).

Received Nov. 27, 1972. P.S.E.B.M., 1973, Vol. 142.