FAT TRANSPORT THROUGH THE LYMPH SYSTEM IN FASTING AND PHLORHIZIN POISONING

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It is an old observation in physiology that ingested fat after absorption by the bowel is transported through the thoracic duct into the blood. The transport of body fat in hunger, certain experimental poisonings, and toxemias is thought to be effected by the blood system, the fat of the fat depots being taken up directly into the capillary blood vessels. The efforts of many investigators to study the transport of body fat in experimental conditions by analysis of the blood fat have yielded few conclusive facts, although the methods have been improved considerably. In fasting, for instance, when body fat is presumably transported through the blood system definite changes in the fat content of the blood cannot be demonstrated. Leathes and Raper (1) explain this by stating that “active transportation of fat in the blood need not mean an increased amount of fat in the blood at any moment,” since “the amount of fat in the blood depends necessarily on two factors: the rate at which additional fat enters and the rate at which it leaves.” The faster the reserve fat is thrown into the blood, the faster it will be removed by the hungry cells; this regulation seems to be sufficiently delicate to prevent marked changes in the blood fat content. The fact that in fasting the mobilized reserve fat cannot be demonstrated in the blood might be explained, however, on a different assumption. It has been claimed recently by several investigators, Hoffmann and Wertheimer (2) and Schur and Löw (3), that the reserve fat in fasting is not conveyed as such at all, but that it is transformed into glycogen and sugar in the fat depots and that this sugar is then transported by the blood to the tissue cells.
Fat in Lymph System

It occurred to us that if reserve fat, when mobilized in fasting is transported as fat, it might be carried to some extent at least by the lymph system. If this assumption is correct, it should be easy to demonstrate considerable fat in the lymph obtained from the thoracic duct of a fasting animal, since the fat content of this lymph would depend only on the rate of the fat mobilization, the absorption of this fat by the hungry tissues being entirely eliminated.

EXPERIMENTAL

In one series of experiments, dogs which had been fasted from 36 hours to 14 days were used. In the second series, the fasting animals were poisoned with phlorhizin. 1.5 to 3.0 gm. of phlorhizin dissolved in 15 to 30 cc. of warm 2 per cent NaHCO₃ were injected subcutaneously on the 3rd and 4th days of fasting, and the dog was operated on on the 5th day. In the early experiments, morphine and ether were used for anesthesia, but were later replaced by nembutal (Abbott), 30 mg. per kilo intravenously, which served very satisfactorily. The thoracic duct was cannulated and samples of lymph were collected in tubes containing potassium oxalate. In some experiments, a cervical lymph vessel was also cannulated and cervical lymph collected. Venous blood was drawn at the same time. The lymph and blood plasma were analyzed for total fatty acids and cholesterol by Bloor's oxidation-titration method (4). Sugar was determined in the oxalated blood and lymph by the Folin (5) modification of the Folin-Wu sugar method. Chylomicron counts were made on the lymph and blood, the dark-field microscope being used in the manner described by Gage and Fish (6).

Results

Fasting—The lymph obtained in all cases was more or less opalescent and contained a few lymphocytes. The chylomicron count of the lymph varied between 75 and 200, while the blood at the same time showed never more than 1 or 2.

As is evident from Table I fasting lymph always contains fat, sometimes in quite considerable amounts. It appears that the amount of fat contained in fasting lymph depends only partly on the duration of the fast, the state of nutrition and the age of the animal being, among other factors, instrumental.
Our finding that the lymph contains considerable fat even on the 2nd day of fasting seems to be at variance with statements of earlier investigators (7), that 24 hours after a fat meal the thoracic duct lymph contains only traces of fat. However, in this early work the fat was determined by extracting the dried lymph with ether. It is known (8) that in body fluids containing large amounts of protein only part of the total fat—the "free" fat—is
determined this way; the alcohol-ether extraction in Bloor's method brings out the fat combined with protein also.

The marked opalescence of fasting lymph and the high number of chylomicrons present suggest that a considerable part of the fat might be in the "free" form. Analysis showed that the fat extracted from dried lymph of fasting animals by ether amounts to about one-half of the total fat extractable by alcohol-ether (in one

### Table I

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Days fasted</th>
<th>Total fatty acids</th>
<th>Cholesterol</th>
<th>Sugar</th>
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<th>Cholesterol</th>
<th>Sugar</th>
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<tr>
<td></td>
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<td>mg. per 100 cc.</td>
<td>mg. per 100 cc.</td>
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<td>59.5</td>
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*Cervical lymph.
† Ether anesthesia.
case 290 mg. as against 540 mg.). No efforts were made at this time to identify the individual lipids or fatty acids and their proportions in the lymph.

The fat content of fasting lymph is usually higher than that of the blood drawn at the same time. The cholesterol content is less, occasionally much less, than that of the corresponding blood. The ratio cholesterol : fatty acids, varies from 1:3 to 1:15 in the fasting lymph, and from 1:2 to 1:6 in the fasting blood.

The glucose content of fasting lymph is always considerably less than that of the corresponding blood. It is also less than in normal lymph, which is known to contain as much sugar as the blood (9).

*Phlorhizin Poisoning*—All animals exhibited marked glycosuria on the day of the operation. The lymph in these cases was usually more opalescent than the fasting lymph, and sometimes resembled skim milk. The cervical lymph obtained in one case, Dog 75, was also quite milky. The sediment and chylomicron counts of the phlorhizin lymph were similar to those of the fasting lymph.

The data in Table II show that the lymph in phlorhizin poison-
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ing contains considerable amounts of fat, usually more than during fasting of similar duration. The cervical lymph contained even more fat than the corresponding thoracic duct lymph. With one exception, Dog 71, the fat content of the lymph was higher than that of the corresponding blood.

The fat content of the blood was rather high in three of the phlorhizin-injected dogs, Dogs 70, 71, 73; in the other three, it was within normal limits although higher than in most of our fasting dogs. The cholesterol content of the lymph was in each case much less than that of the corresponding blood, but usually higher than that of fasting lymph. Most of the phlorhizin-injected dogs exhibited hypercholesterolemia. The ratio, cholesterol : fatty acids, varied from 1:6 to 1:16 in the phlorhizin lymph, and from 1:2 to 1:4 in the blood.

The sugar content of the phlorhizin blood was low, but that of the phlorhizin lymph was still lower.

DISCUSSION

The possibility of the presence in the lymph of substances other than fatty acids of high molecular weight titratable by Bloor's method must be considered. Lactic acid and especially β-hydroxybutyric acid might be present in the lymph under the experimental conditions. However, they are soluble in water and practically insoluble in petroleum ether, and would therefore not appear in the final extractions. Acetone and diacetic acid, if present, would be entirely eliminated during the repeated processes of evaporation. The anesthetic used, nembutal (pentobarbital sodium), is insoluble in petroleum ether. Qualitative tests easily demonstrated the presence of fat in the lymph.

Where does the fat found in the lymph during fasting come from? It might be blood fat filtered through the blood capillaries directly into the lymph spaces and lymph vessels. However, the fact that the lymph usually contains much more fat than the blood, while at the same time it always contains much less sugar and cholesterol, indicates that at least part of the lymph fat must come from sources other than the blood. Another suggestive evidence of this is our finding of chylomicrons in the lymph in fasting, with none in the blood.
To settle this point definitely, we examined the fat content of cervical lymph at the height of alimentary lipemia. After 24 hours fasting, two dogs were fed each \( \frac{3}{4} \) pint of cream and four egg yolks. 4 hours later the cervical lymph duct was cannulated and lymph collected for 1 hour. Immediately following this, the thoracic duct was cannulated. The clear cervical lymph contained 200 mg. and 210 mg. of fat per 100 cc., respectively, while the milky thoracic duct chyle contained 1580 mg. and 4500 mg. of fat per 100 cc. In these dogs evidently there was movement of considerable fat through the thoracic duct into the blood and from the blood into the tissues; yet the cervical lymph at the same time contained very little fat. This shows clearly that blood fat does not directly enter the lymphatics even when it leaves the blood stream rapidly and in large amounts.

We must conclude then that the lymph fat in fasting comes chiefly from the reserve fat of the depots and tissues. We have not as yet attempted to determine which tissues are the chief sources of this mobilized fat. However, it is evident from our data that the liver and other abdominal organs are not exclusive sources since the cervical lymph may also contain considerable fat.

What is the physiological importance of the fat transport through the lymph system in fasting? On calculating the 24 hour amount of the lymph flow in fasting at about one-sixteenth (this is an estimate from lymph fistula dogs) of the body weight (10), it is evident that the total amount of fat transported this way is but a fraction of the total fat consumption, probably not more than 20 per cent. The remainder of the mobilized fat is then either taken up by the blood capillaries of the fat depots and transported by the blood system directly, or it is transformed in the fat depots into sugar and transported as sugar. It is also possible that both these processes are taking place at the same time. However, our finding that both in fasting and phlorhizin poisoning, the lymph contains less sugar than normally, speaks strongly against the transport of mobilized fat in the form of sugar. Accordingly, most of the reserve fat must enter the blood capillaries, at a rate proportional to that at which it enters the lymph system.

We believe that the fat content of fasting lymph offers a reliable index of fat mobilization. It is possible to study the rate of fat
mobilization under various experimental conditions by analyzing the fat content of the lymph.

SUMMARY AND CONCLUSIONS

1. The lymph contains considerable amounts of fat in fasting and in phlorhizin poisoning. There is more fat but less cholesterol than in the corresponding blood.
2. The sugar content of fasting and phlorhizin lymph is always considerably less than that of the corresponding blood, and also less than that of normal lymph. This finding speaks strongly against the transport of mobilized fat in the form of sugar.
3. Most of the fat in fasting lymph is mobilized reserve fat.
4. Fat transport through the lymph system offers new possibilities to study the phenomena of fat mobilization.

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