

Effects of intravenous heparin on oxidation of fat

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[Received for publication September 22, 1959]

SUMMARY

The intravenous injection of heparin to carbohydrate-fed rats results in an elevation of plasma free fatty acid levels and in a lowering of the respiratory quotient from 1.0 to 0.9. The injection of heparin simultaneously with palmitic acid-C¹⁴-labeled chylomicrons results in an elevation of the specific radioactivity of the expired CO₂. These observations suggest that heparin causes an obligatory oxidation of fat, presumably through the release of free fatty acid from triglyceride.

It has been repeatedly observed in a variety of mammalian species, including man, that the intravenous injection of heparin is followed immediately by the appearance in the blood stream of a "clearing factor." This factor is apparently identical with the enzyme "lipoprotein lipase" isolated by Korn and Quigley (1) from adipose tissue. It characteristically hydrolyzes the triglycerides contained in chylomicrons and low-density lipoproteins with the accumulation, at least *in vitro*, of free fatty acids (FFA). In plasma, the latter are "free" in the sense that they are not in covalent linkage, but they are, for the most part, tightly bound to albumin.

It has been demonstrated by several groups (2, 3) that FFA leave the blood stream more rapidly than do chylomicrons, although the half life of the latter is a matter of only a few minutes, dependent to some extent on the dose (4). If it is assumed that the first step in the oxidation of triglycerides is a hydrolytic one to form the free fatty acids, then one might expect the injection of heparin to have some effect on the oxidation of triglyceride fat.

French and Morris (5) have already demonstrated in fed rats injected with palmitic acid-C¹⁴-labeled chyle that heparin did indeed increase the amount of C¹⁴O₂ excreted, particularly in the first hour. They did not determine the respiratory quotient. The present experiments were designed to confirm the findings of French and Morris and to extend them.

METHODS

Two different types of experiment were designed to elucidate the role of heparin in the oxidation of fat. In

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the first type of experiment the specific activity of the expired CO₂ was measured following the injection of palmitic acid-C¹⁴-labeled rat chylomicrons or labeled FFA, with and without heparin. In the second type of experiment, the respiratory quotients (RQ) of carbohydrate-fed rats were determined with and without heparin injection.

Male Sprague-Dawley rats weighing between 175 and 300 g. were used. They were housed in individual cages. Twenty-four hours before an experiment they were offered only sugar tablets to eat and their drinking water was replaced with a solution of 10 per cent sucrose in 0.5 per cent NaCl. All rats subsequently used had eaten 5 to 10 g. of sugar and had drunk 50 to 125 ml. of the sucrose-saline solution. In addition, 30 minutes before an experiment each animal received 1 or 2 ml. of 50 per cent sucrose by stomach tube. All injections were given by tail vein. Experimental animals received 1 ml. of physiologic saline containing 0.25 mg. heparin; control animals received the saline only.

When the specific activity of the expired CO₂ was being measured, carbohydrate-fed rats received either heparin or saline intravenously, followed by 1 ml. of labeled chylomicrons or FFA. The chylomicrons were prepared by methods previously described (6) from chyle obtained from a cannulated rat which had previously been fed olive oil containing a trace amount of palmitic acid-1-C¹⁴. Previous experience (2) has shown that 97 per cent of the chylomicron label is in the triglycerides. The preparation of FFA was made by the method previously described (3). It contained palmitic acid-1-C¹⁴ bound to bovine serum albumin and dissolved in Krebs-Ringer solution buffered at pH 7.2. Following injection of the label, the animal was immediately placed in a train with the

TABLE 1. EFFECT OF HEPARIN ON THE $C^{14}O_2$ EXCRETION AND ON THE SPECIFIC ACTIVITY OF THE CO_2 AFTER THE INJECTION OF C^{14} -LABELED CHYLOMICRONS OR C^{14} -LABELED FREE FATTY ACIDS (FFA)

| No. of Rats | Treatment | $C^{14}O_2$ | Significance of Difference | | S.A. of CO_2 | Significance of Difference | |
|-------------|----------------------------|---------------------------------------|----------------------------|------|---------------------------------|----------------------------|----------|
| | | | t | p | | t | p |
| 8 | C^{14} -Chylos + saline | per cent of dose $1.30 \pm 1.25^*$ | | | cpm./mmole $1400 \pm 1195^*$ | | |
| 8 | C^{14} -Chylos + heparin | 4.42 ± 3.62 | 2.15 | 0.05 | 5039 ± 3462 | 2.51 | 0.025 |
| 9 | C^{14} -FFA + saline | 9.44 ± 6.23 | | | 776 ± 496 | | |
| 9 | C^{14} -FFA + heparin | 5.28 ± 3.08 | 1.69 | 0.1 | 506 ± 265 | 1.89 | 0.05-0.1 |

* Standard deviation.

following characteristics: Room air was drawn through soda lime and then through a cylindrical glass chamber just large enough to accommodate the rat comfortably. Moisture was condensed out by passing the air around a container of solid CO_2 in ethanol. The dry air was bubbled through sintered glass into 100 ml. of a solution of about 0.25 N Hyamine in methanol, as devised by Fredrickson and Ono (7). A final $Ba(OH)_2$ trap indicated that no CO_2 escaped through the Hyamine. Collections continued for 30 minutes, after which time 5 ml. aliquots were titrated in triplicate with 0.25 N HCl and 2 ml. aliquots were counted in duplicate in a liquid scintillation counter after the addition of 15 ml. of a 0.5 per cent solution of diphenyloxazole in toluene.

When the respiratory quotient was being measured, the animal was placed in a closed-circuit train through which CO_2 -free room air was circulated. A pump circulated the air through the animal chamber, a CO_2 absorbant (100 ml. of 0.5 N KOH), a spirometer of 6 l. volume, and the pump. The animal remained in the train for 1 hour following injection. Oxygen consumption was read directly from the spirometer scale with a maximum error of 3 per cent. Excreted CO_2 was measured by titration of the KOH with 0.5 N HCl in triplicate samples. Individual titrations were within 2 per cent of the mean. The experiments were performed in an air-conditioned room and it was assumed that during the collection temperature and atmospheric pressure did not vary significantly.

It has been shown by Grossman *et al.* (8) that the intravenous injection of heparin in fasting rats is followed by an increase in the FFA content of the blood. It was necessary, however, to demonstrate the same phenomenon in carbohydrate-fed animals. Six-

teen such rats were divided into two groups, one of which received heparin in saline and the other saline only.

Fifteen minutes later each animal was anesthetized with intravenous pentobarbital and 5 ml. of blood was rapidly withdrawn from the aorta into a syringe containing a small amount of dry heparin. The blood was immediately transferred to centrifuge tubes in a bath of ice water. The blood was centrifuged at $0^\circ C$ and 1 ml. of plasma extracted in duplicate. The FFA were determined by the method of Dole (9). We had previously determined, as had Grossman *et al.* (8), that incubation of rat blood in ice water completely inhibited the activity of lipoprotein lipase. It was necessary to do this to prove an *in vivo* effect of heparin on FFA levels.

RESULTS

The results of the experiments in which labeled chylomicrons or FFA were injected with and without heparin are presented in Table 1. Following the injection of chylomicrons, heparin more than tripled the amount of $C^{14}O_2$ expired, as well as tripling the specific activity of the CO_2 . Following the injection of labeled FFA, there was an actual decrease both in the amount of $C^{14}O_2$ and in the specific activity of the CO_2 . Although not statistically significant ($p = 0.05-0.1$), this probably represents a dilution of the circulating isotope caused by the elevation in plasma FFA brought about by heparin, as is shown in Table 2.

In addition to showing a significant elevation in plasma FFA levels following the injection of heparin, Table 2 also shows that heparin lowers the respiratory quotient. In one experiment, 11 rats that received saline are compared with 10 rats that received he-

TABLE 2. THE SERUM FREE FATTY ACID (FFA) LEVELS AND THE RESPIRATORY QUOTIENTS OF CARBOHYDRATE-FED RATS INJECTED WITH SALINE OR HEPARIN

| No. of Rats | Treatment | FFA | RQ | Significance of Difference | |
|-------------|-----------|----------------------------------|----------------|----------------------------|--------|
| | | | | t | p |
| 8 | Saline | <i>meq./l.</i> 0.17 ± 0.033 * | | | |
| 8 | Heparin | 0.24 ± 0.048 | | 3.03 | <0.01 |
| 11 | Saline | | 0.97 ± 0.070 * | | |
| 10 | Heparin | | 0.87 ± 0.065 | 3.22 | <0.01 |
| 10 † | Saline | | 1.02 ± 0.08 | | |
| 10 | Heparin | | 0.90 ± 0.09 | 4.20 | <0.005 |

* Standard deviation.

† Saline and heparin injected into same animals.

parin. In a second experiment, 10 rats served as their own controls; the RQ was determined first after saline and then after heparin. In the interval between these measurements, additional carbohydrate was given by stomach tube. In both experiments the RQ of the animals receiving saline approached unity, indicating that their energy requirements were being met primarily through the oxidation of carbohydrate. In both experiments there was a highly significant decrease in the RQ after the injection of heparin.

DISCUSSION

As pointed out by French and Morris (5), who demonstrated an increased rate of excretion of $C^{14}O_2$ in rats receiving labeled chyle and heparin as compared to saline controls, the effect is probably explained by the observation that FFA is more readily available for oxidation than is triglyceride. The injection of heparin with chylomicrons, through the release of lipoprotein lipase, permitted a more rapid conversion of the triglyceride to FFA. This interpretation is strengthened by the observation that when the label was injected as FFA, heparin had no such effect. It should not be inferred from these findings that retransport of chylomicron triglyceride as plasma FFA is an obligatory step preceding oxidation. That some chylomicrons may be removed from the blood and oxidized

without recycling as plasma FFA has been emphasized by Fredrickson *et al.* (10).

Of more interest is the observation that heparin will lower the RQ of carbohydrate-fed rats. The injection of heparin apparently results in an obligatory oxidation of fat, even in the presence of adequate amounts of carbohydrate. One can only speculate on the mechanism involved here, but it seems not unlikely that the release of lipoprotein lipase causes the hydrolysis of adipose or other tissue triglyceride, and that the accumulation of FFA, as reflected in an elevation of plasma FFA, brings about an obligatory oxidation.

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