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Changes in the lipolytic activity of the guinea pig mammary gland at parturition

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SUMMARY

During pregnancy, the clearing factor lipase activity of the guinea pig mammary gland remains constant until immediately before parturition. It then increases rapidly until, at parturition, the activity per gland is some 100 times greater than during pregnancy. Activity remains at this high level after parturition. If suckling is prevented at one of the two mammary glands of the guinea pig by tying off the teat, the activity of that gland falls within a few days to a level that is only some 5 times greater than that in the gland during pregnancy. The activity of the suckled gland remains high. The activity of lactating mammary gland tissue is much higher than that of any other tissue studied hitherto.

It has been suggested that the passage of chylomicron triglycerides from the bloodstream into tissues other than the liver might be facilitated by their hydrolysis to free fatty acids (FFA), which are known to leave the blood extremely rapidly. This hydrolysis is believed to be due to the action of the enzyme clearing factor lipase (lipoprotein lipase)[1] acting at a site close to the blood capillary wall (1, 2).

Preliminary results of studies still in progress[2] have suggested that chylomicron triglyceride is removed from the bloodstream by the mammary gland during lactation, presumably to contribute to the milk fat. If this uptake involves hydrolysis of the triglyceride by the clearing factor lipase, then the activity of this enzyme in the mammary gland should rise when lactation begins. The following experiments suggest that this is so.

[1] The lipase released into the blood after the injection of heparin, known as "clearing factor lipase" (1), and that first studied by Korn in extracts of heart and adipose tissue, called by him "lipoprotein lipase" (3), have many characteristics in common and are presumed here to be the same enzyme. The mammary gland lipase studied in this paper has the same properties as the clearing factor, or lipoprotein lipase, and therefore could be referred to by either of these terms. For reasons stated elsewhere (1), "clearing factor lipase" is preferred by this author. This is the same enzyme as the "lipoprotein lipase" described by McBride and Korn (see p. 17).

[2] J. M. Barry, W. Bartley, J. L. Linzell, and D. S. Robinson, unpublished observations.

METHODS

Female guinea pigs were used. The stage of pregnancy was roughly assessed from the degree of development of the fetuses when the animals were killed.

The clearing factor lipase content of the mammary gland was estimated by the ability of aqueous homogenates of acetone/ether powders of the tissue to hydrolyze chylomicron triglyceride with the liberation of FFA. Hitherto, extracts of tissue acetone powders have been most usually used as a source of the enzyme (3). However, the results of a recent extensive study in this department on the clearing factor lipase of adipose tissue[3] have led to the conclusion that tissue acetone/ether powders have certain advantages over acetone powders. Additional lipid is removed from the tissue residues without loss of enzyme activity by further extraction with ether, and a greater proportion of the total enzyme of the tissue is extractable from acetone/ether powders than from acetone powders in a soluble form. In the present experiments, approximately 66% of the total lipase activity was found in the supernatant solution when aqueous homogenates of acetone/ether powders were centrifuged for 30 min at 2500 rpm. A similar distribution of the enzyme is found with homogenates of acetone/ether powders of adipose tissue whereas,

[3] M. R. Salaman, unpublished observations.

TABLE 1. THE CLEARING FACTOR LIPASE ACTIVITY OF HOMOGENATES OF ACETONE/ETHER POWDERS FROM GUINEA PIG MAMMARY GLAND

Estimated Days Pre-Partum	Days Post-Partum	Wt of Gland (g)	Lipase activity*	
			μmoles FFA released/gland/hr	μmoles FFA released/g gland fresh wt/hr
7		1.6	67	41
4		3.4	176	52
3		3.8	128	34
2		3.0	71	23
2		4.9	500	102
1		6.8	1,900	280
	1	5.0	11,800	2,400
	2	12.8	16,800	1,310
	2	13.3	19,600	1,470
	2	13.0	29,500	2,250
	5	10.5	15,400	1,460
Nonpregnant		1.0	88	88
Nonpregnant		0.8	11	14

* Each value represents a result of the analysis of the mammary gland tissue from a single animal.

TABLE 2. IDENTIFICATION OF THE MAMMARY GLAND ENZYME AS THE CLEARING FACTOR LIPASE*

Additions or Omissions	Activity	
	Nonpregnant gland	2 days post-partum gland
	%	%
With 0.4 M NaCl	5	8
With protamine sulphate (4 mg/ml)	8	10
Without heparin	42	18
With chyle in the place of the chyle-serum mixture	65	62

* Two enzyme preparations were used, one from the mammary tissue of a nonpregnant guinea pig and the other from the tissue of a guinea pig 2 days post-partum. Enzyme assays were carried out under the conditions described in Methods but with the additions or omissions shown. Activities are reported as percentage of the enzyme activity as measured in the complete system.

with acetone powers of adipose tissue, only 33% of the total lipase activity is extractable (4).

The entire secretory tissue of one of the two mammary glands of the guinea pig was used in each experiment. While the animal was under anesthesia, the gland was dissected and washed in 0.9% (w/v) sodium chloride solution, dried on filter paper, and weighed. No attempt was made to remove the milk from those glands taken after parturition and their weights include the contained milk. Some adipose tissue was inevitably taken with the secretory tissue. For this reason, the clearing factor lipase activity of samples of guinea pig adipose tissue taken from beneath the abdominal wall was also measured.

After coarse mincing with scissors, the tissue was homogenized at 4° in a Waring blendor in 250 ml of acetone. The homogenate was filtered at 4° on a Buchner funnel and the precipitate was washed at room temperature successively with 300 ml of acetone and 200 ml of ether. The last traces of ether were removed *in vacuo*. Known weights of the dry powder were homogenized at 4° in a Potter-type glass homogenizer with a Teflon plunger in 0.025 N ammonia solution adjusted to pH 8.5. Between 0.25 and 0.5 mg of powders from the mammary gland tissue obtained post-partum and between 1 and 5 mg of powders from the mammary gland tissue obtained pre-partum and from adipose tissue were homogenized per milliliter of ammonia solution. Samples (3 ml) of the homogenates were taken immediately and added to incubation flasks containing 2.4 ml of 20% (w/v) bovine albumin (Fraction V; Armour Pharmaceutical Co., Ltd.) in Krebs-Ringer solution of twice the normal strength at pH 8.1, 0.1 ml 40 unit/ml heparin (100 units/mg, Pularin-Evans), 1 ml 1.35 M tris(hydroxymethyl)aminomethane buffer at pH 8.1, and 1.8 ml of a serum-chyle mixture. Water or other additions (see Table 2) were added to a final volume of 9 ml. The flasks were incubated with shaking at 37° and 1-ml samples of the contents were taken in duplicate initially and at 1 hr or 2 hr thereafter for the determination of FFA by the method of Dole (5) as modified by Salaman and Robinson (4). These assay conditions are those developed for the clearing factor lipase of acetone/ether powders of adipose tissue[3] and the rate of FFA release is constant during the incubation period.

The chyle was collected from the thoracic ducts of rats fed 1 ml of olive oil. The serum was prepared by adding 1 vol of 1 M $CaCl_2$ solution to 40 vol of citrated rat plasma and removing the clot that formed after 30 min at 37°. The techniques of collection of chyle and citrated plasma have been described (6). The serum-chyle mixture was prepared by adding 1 vol of rat chyle to 4 vol of rat serum. The mixture was left for 1 hr at room temperature before being added to the incubation system. It contained between 10 and 16 mg chyle triglyceride fatty acids/ml.

RESULTS

The data in Table 1 show that the lipase activity of the guinea pig mammary gland is relatively constant throughout pregnancy but that there is a large increase

in activity beginning immediately prior to parturition and persisting during lactation. The lipase content of the gland during lactation is approximately 100 times its content during pregnancy.

In one experiment, the teat of one of the two mammary glands of a guinea pig was tied off one day before parturition. The lipase activities of each gland were then determined 3 days after parturition when the young had been suckling from only one gland. In the suckled gland (weight 10.5 g), the lipase activity was equivalent to a release of 15,400 μmoles FFA/gland/hr or 1460 μmoles FFA/g gland fresh weight/hr. In the tied-off gland (weight 4 g), the activity was 400 μmoles FFA/gland/hr or 102 μmoles FFA/g gland fresh weight/hr. Thus, suckling and consequent continued milk production by the gland appears to be a factor in maintaining the high lipase activity.

That the clearing factor lipase is the lipase in the gland that is responsible for the FFA release is demonstrated by the properties of the enzyme shown in Table 2. Its activity is inhibited almost completely by 0.4 M NaCl solution and by protamine sulphate and, in the absence of added heparin, the activity is markedly reduced. The ability of serum to increase the rate of hydrolysis of chylomicron triglyceride by the enzyme is a further characteristic (4).

Acetone/ether powders of guinea pig adipose tissue were prepared. Their lipase activity was equivalent to a release of between 50 and 100 μmoles FFA/hr/g tissue fresh weight. This is approximately the activity of the guinea pig mammary gland tissue during pregnancy.

DISCUSSION

It is suggested that the large increase in the clearing factor lipase activity of the mammary gland at parturition might be related to an uptake of chylomicron triglyceride from the blood in the lactating animal (see introduction). If so, it provides a second example of changes in tissue clearing factor lipase activity occurring in parallel with changes in chylomicron triglyceride uptake. Earlier studies in the rat have shown that the decreased uptake of chylomicron triglyceride fatty acid by adipose tissue during fasting (7) is accompanied by a fall in the clearing factor lipase activity of the tissue (4, 8, 9, 10, 11). These changes, therefore, are consistent with the view that the distribution of clearing factor lipase activity in the extrahepatic tissues might determine the sites other than the liver in which triglyceride fatty acids leave the blood (1, 2).

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