Effect of fasting on the clearing factor lipase (lipoprotein lipase) activity of fresh and defatted preparations of rat heart muscle

J. BORENSZTAJN,* S. OTWAY, and D. S. ROBINSON

Department of Biochemistry, University of Oxford, Oxford, England

ABSTRACT The clearing factor lipase (lipoprotein lipase) activities of homogenates of fresh tissue and of acetone-ether powders have been compared in hearts from fed and starved rats.

The activity of the enzyme measured in homogenates of acetone-ether powders is generally higher than that in homogenates of the fresh tissue. Activation is due to an effect of the acetone-ether treatment on enzyme which is associated with the tissue residue in fresh tissue homogenates. A similar activation occurs when the tissue residue is treated with deoxycholate.

When rats are fasted, a marked increase in the clearing factor lipase activity of the heart occurs. Peak activities are reached after 10–24 hr, and thereafter the activity falls slowly. This pattern of activity is observed in homogenates of fresh tissue and of acetone-ether powders. The activity of clearing factor lipase in diaphragm muscle also increases in rats starved for 8 or 24 hr.

The importance of the change in muscle clearing factor lipase activity on fasting in relation to triglyceride fatty acid utilization by this tissue is emphasized.

SUPPLEMENTARY KEY WORDS triglyceride fatty acid utilization · skeletal muscle

THE RESULTS OF published studies on the clearing factor lipase activity of the rat heart are difficult to compare because two types of preparations have been used to assay the total enzyme activity of the tissue. Korn (1, 2) first studied the enzyme in extracts of defatted powders of heart muscle, but further work on such preparations showed that not all the enzyme was solubilized under the conditions which he described. This led to the use of homogenates of the powders for assays of the total enzyme activity (3, 4). A second preparation which has been used is a homogenate of the fresh tissue (5–12). However, the various studies with such homogenates are difficult to compare because a variety of homogenizing media has been used and because conditions of assay have differed.

These differences could explain why some investigators have found an increase in the activity of the enzyme in rats starved for 48 hr and longer (5, 6, 8), while other workers have observed a decrease in activity in animals starved for 48 hr (4). In the latter study, the enzyme was assayed at pH 8.1 in homogenates of defatted tissue powders while, in the others, it was assayed at pH 7.4 in aqueous homogenates of the fresh heart tissue.

In the present paper, the clearing factor lipase activities of homogenates of fresh tissue and of acetone-ether powders of the rat heart are compared under the same assay conditions, and the results of a reinvestigation of the effect of fasting on the activity of the enzyme are reported.

MATERIALS AND METHODS

Female rats of the Wistar strain, weighing 180–200 g and maintained on Oxoid pasteurized breeding diet, were used. This diet contained approximately 50% carbohydrate, 20% protein, 5% fat, and 25% moisture, minerals, and undigestible matter. Fed animals are defined as rats which had been fed this normal diet and which were killed between 8 a.m. and 9:30 a.m. Starved

Abbreviations: FFA, free fatty acids; TGFA, triglyceride fatty acids.

* Present address: Department of Physiology, University of Chicago, Chicago, Illinois 60637.

animals are defined as rats which, after being maintained on the normal diet for several weeks, were starved for different periods of time starting from either 8 a.m. or 9 a.m. on the first day of the experiment. In some experiments, rats with access to food were killed at times later in the day than 9:30 a.m. These animals are described as rats on the normal diet. In other experiments, rats were starved from 8 a.m. and while under ether anesthesia, force-fed by stomach tube at 12 noon and at 5 p.m. with glucose, olive oil, or a combination of these with casein hydrolyzate. Details are reported with the individual experiments.

Tissue Preparations

Two main types of tissue preparation were used: homogenates of fresh heart muscle, and homogenates of acetone-ether powders prepared from homogenates of fresh heart muscle.

Homogenates of Fresh Heart Muscle. These were prepared from hearts immediately after their removal from rats which had been anesthetized with ether. Only the ventricles were dissected out; they were rinsed in 0.9% NaCl, lightly blotted, and weighed on a torsion balance. After coarse mincing with scissors, portions were homogenized at 4°C using a Potter–Elvehjem type homogenizer with a motor driven Teflon pestle. As a standard procedure, 12 full strokes of the pestle were used. All hearts were homogenized at a tissue concentration of between 20 and 30 mg (fresh weight) per ml of homogenate. The usual homogenizing media were 0.025 N NH_3–NH_4Cl buffer (pH 8.1), or rat serum (pH 8.0), with or without heparin at a final concentration of 1 IU/ml. The serum was prepared from recalcified citrated plasma as previously described (13). Where other homogenizing media were used, details are given with the individual experiments.

In some experiments homogenates were centrifuged for 30 min at 105,000 *g* at 0°C in a Spinco model L preparative ultracentrifuge (No. 50 rotor) in order to separate enzyme in solution from enzyme associated with the tissue residue. The supernatant solution was recovered, and the tissue residue was redispersed at the original homogenate concentration. Where required, centrifugation and redispersion were repeated.

Control studies showed that the clearing factor lipase activity of homogenates of fresh heart tissue, was stable for at least 2 hr at 0°C and assays were always started within this time.

Homogenates of Acetone-Ether Powders. Acetone-ether powers were usually made from homogenates of the fresh tissue in serum (20–30 mg of tissue [fresh weight] per ml), prepared as described above. Samples of between 5–8 ml of the fresh tissue homogenate were mixed with 200 ml of acetone at 4°C. The precipitated material was filtered on a Büchner funnel and washed with 200 ml of acetone and 200 ml of ether at room temperature (3). After storage in vacuo for at least 1 hr, the dry preparation was finely minced with scissors, and portions (equivalent to 40–60 mg of fresh tissue) were homogenized in 10 ml of 0.025 N NH_3–NH_4Cl (pH 8.1) as previously described (4). In some experiments, the acetone-ether dried preparations were stored overnight in vacuo before samples were taken for homogenization. Less than 5% of the activity of the preparations was lost under these storage conditions. Control experiments showed that the activity of the enzyme in the homogenates was stable for at least 2 hr at 4°C, and assays were always started within this time.

Acetone-ether dried powders were also prepared from homogenates of the fresh tissue in water, in 0.025 N NH_3–NH_4Cl (pH 8.1), and in 5% (w/v) albumin in 0.025 N NH_3–NH_4Cl (pH 8.1), and, in one experiment. the fresh tissue was homogenized directly in acetone at 4°C.

In some experiments acetone-ether powders were prepared from diaphragm muscle. A similar technique was employed except that a Silverson homogenizer (Silverson Machines Ltd., London, England), fitted with a microtubular head, was used for the initial disruption of the muscle in serum (50 mg tissue [fresh weight] per ml).

Clearing Factor Lipase Assay

Clearing factor lipase activity is expressed in terms of the quantity of FFA released at 37°C from a suitable triglyceride substrate, usually chyle triglyceride which has been activated by preincubation with serum (3). 1 unit of activity represents the release of 1 μmole of FFA during incubation for 1 hr, and activities are expressed as units/g of tissue (fresh weight). Expression as units of activity per heart does not affect the significance of the findings. Where at least five observations are made, the results are expressed as means ±SD. The probability (*P*) of the significance of the difference between means was tested by applying Behren's modification of Students "t" test (14).

The assay medium was slightly modified from that described previously (13) and had the following composition: 2 vol of 20% (w/v) albumin in water (pH 8.1), 1 vol of 0.7 M Tris–HCl (pH 8.1), 0.5 vol of chyle containing 100–150 μeq of TGFA/ml, 0.5 vol of rat serum, and 0.5 vol of heparin solution (14 IU/ml). In most experiments this assay medium was prepared and preincubated for 30 min at 37°C before portions (4.5 ml) were distributed into the assay flasks. Then samples (2.5 ml) of the enzyme preparation were added, and the whole assay mixture was incubated in a water bath with shaking at 37°C for 1–3 hr, according to the activity of the prepara-

tion. This procedure was modified in some cases, and details are given with the individual experiments.

Previous studies (3) suggested that the amount of serum used was sufficient for maximum activation of the chylomicron triglyceride. Since the enzyme preparation was itself present in a serum medium in some assays, this point was confirmed in the present study.

Heparin was normally present in the assay mixture at a final concentration of 1 IU/ml. In some experiments, the enzyme preparation contained heparin, and the final concentration in the assay was then somewhat greater than 1 IU/ml. However, variations in concentration over the range 1–5 IU/ml had no effect on enzyme activity.

Samples (1 ml) of the assay mixture were taken in triplicate at the beginning and end of the incubation for FFA determination by the method of Dole and Meinertz (15), as modified by Salaman and Robinson (13). Tetra-*n*-butyl ammonium hydroxide (5 mM) in methanol was the titrant (16). Under these conditions, no more than 20% of the triglyceride substrate was hydrolysed, and the release of FFA was linear throughout. Control assays, in which the enzyme preparation was replaced by the medium in which it was dissolved or suspended, were always carried out, and the small amount of FFA which was released was subtracted from the FFA released in the assays with enzyme present.

In experiments in which the lipase was characterized, the serum–chyle mixture in the assay medium was replaced by a triglyceride emulsion (Intralipid) at an equivalent TGFA concentration. Serum was sometimes included; when it was omitted it was replaced by 0.9% sodium chloride solution.

Heparin (1000 IU/ml, Pularin) was obtained from Evans Medical Supplies Ltd., Liverpool. Sodium deoxycholate was obtained from British Drug Houses, Ltd. (Poole, England), and Triton X-100 from Nuclear Enterprises (G.B.) Ltd., Edinburgh. The albumin was bovine serum albumin (Fraction V; Armour and Company, Ltd., Eastbourne, England). Intralipid was obtained from Vitrum (Stockholm, Sweden). The pH of both NH_3–NH_4Cl and Tris buffers showed considerable temperature dependence. All the pH values quoted are based on measurements made at approximately 20°C.

RESULTS

The Clearing Factor Lipase Activity of Fresh and Defatted Preparations of Rat Heart Muscle

Homogenates of Fresh Tissue. When rat heart muscle is homogenized directly in an aqueous medium, part of the clearing factor lipase activity of the homogenate is in solution and part is associated with the tissue residue (7, 8, 10, 17). Iselin and Schuler (17) have shown that, if heparin is present when the heart is homogenized, the percentage of the total activity of the homogenate which is in solution, is increased. These findings have been confirmed in the present study (Expt. A vs. B, and C vs. D, Table 1). In addition, it has been shown that the percentage of enzyme in solution in the homogenate is higher when the homogenates are prepared in serum, than when they are prepared in ammonia buffer (Expt. A vs. C, and B vs. D). Incubation for 15 min at 37°C of homogenates prepared in serum and in the presence of heparin further increases the percentage of the total enzyme activity which is in solution (Expt. D vs. E). These findings have been substantiated in several similar experiments. The effect of heparin is also evident with homogenates prepared in water or in 5% albumin in water (w/v) at pH 8.1. The effects of heparin and of serum are also seen in experiments with hearts from rats starved for 48 hr, as well as with hearts from rats on the normal diet.

In all the experiments reported in Table 1, clearing factor lipase was assayed in the presence of heparin. When homogenates prepared without heparin were assayed for clearing factor lipase in the absence of heparin, lower activities than those reported were obtained. Thus, in Expt. A (Table 1), the activity of the homogenate fell from 118 to 66 units/g of tissue (fresh weight). This effect of heparin in the assay may be explained if the enzyme is either not active at all, or is not fully active when it is associated with the tissue residue (17). If this is so, then

TABLE 1 THE EFFECT OF HEPARIN AND SERUM ON THE DISTRIBUTION OF CLEARING FACTOR LIPASE ACTIVITY IN HOMOGENATES OF FRESH HEART TISSUE

Expt.	Homogenizing Medium	Homogenate Activity	Percentage of Homogenate Activity in: Supernatant	Tissue Residue
		*units of lipase activity/g of tissue**		
A	Ammonia	118	39	66
B	Ammonia + heparin	120	50	53
C	Serum	122	47	57
D	Serum + heparin (0°C)	124	69	34
E	Serum + heparin (37°C)	130	81	26

Hearts from four rats on their normal diet were coarsely minced together, and separate portions of the mince were homogenized in 0.025 N NH_3–NH_4Cl at pH 8.1 or in serum (25 mg fresh tissue per ml), with and without heparin (final concentration, 1 IU/ml). In Expt. A–D, the homogenate was kept for 15 min at 0°C, while in Expt. E it was kept for 15 min at 37°C. Samples of each homogenate were then centrifuged. The supernatant solution was retained in each case, and the tissue residue was redispersed in the appropriate homogenizing medium, at a concentration equivalent to that in the original homogenate. Assays of the clearing factor lipase activity of duplicate portions of the homogenates (kept for 30 min at 0°C), of the supernatant solutions, and of the redispersed residues were carried out as described in Methods.

* Fresh weight.

TABLE 2 SOLUBILIZATION OF CLEARING FACTOR LIPASE DURING ASSAY OF THE ENZYME AT 37°C

Expt.	Time of Incubation of Assay Mixture at 37°C	Clearing Factor Lipase Activity of: Total Assay mixture	Clearing Factor Lipase Activity of: Soluble Fraction of Assay Mixture	Percentage of Activity in Solution in Assay Mixture
	min	*units of lipase activity/g of tissue**		
A	0	—	58	50
	5	—	87	76
	10	—	96	82
	15	116	94	81
B	5	—	85	75
	15	114	94	82

Expt. A. Hearts from two rats on the normal diet were coarsely minced together, and a sample of the mince was homogenized in serum (20 mg of fresh tissue/ml). One portion of the homogenate was mixed with assay medium at 0°C, and a second portion was mixed with assay medium at 37°C. The ratios of homogenate volume to assay medium volume were as described in Methods. Samples of the mixture at 37°C were removed at 5 and 10 min and immediately cooled to 0°C. Two further samples were removed at 15 min and cooled to 0°C. The samples removed at 5 and 10 min, and one of those removed at 15 min, as well as a sample of the mixture kept at 0°C throughout, were centrifuged. The whole of the supernatant solution, together with the layer of assay medium lipid at the surface, was recovered from each centrifuge tube. The lipid was thoroughly dispersed in its supernatant solution, and clearing factor lipase was assayed in each of the resulting mixtures. Enzyme was also assayed in the uncentrifuged sample of the assay mixture removed after 15 min at 37°C.

Expt. B. This was carried out with a further portion of the mince used in Expt. A. The conditions were as described under Expt. A except that the hearts were homogenized in 0.025 N NH_3–NH_4Cl at pH 8.1 and values were obtained for the clearing factor lipase activities of the supernatant fractions of the assay mixture only after 5 and 15 min incubation at 37°C.

* Fresh weight.

the relative constancy of the homogenate activities reported in Table 1, despite the variations in the proportion of the total activity which is in solution in the different media, could be due to the fact that in all cases a high percentage of the enzyme is brought rapidly into solution when it is assayed in the presence of heparin. The results of the experiment described in Table 2 show that, in fact, at least 75% of the total enzyme activity of homogenates of fresh tissue, prepared in either serum or in 0.025 N NH_3–NH_4Cl at pH 8.1, is in solution within 5 min of setting up assays at 37°C.

It is not clear whether heparin also affects the activity of enzyme in solution. Iselin and Schuler (17) could find no such effect while Gartner and Vahouny (10) reported that under certain conditions there was an approximate doubling of the activity of enzyme in solution when heparin was present in the assay. Under the conditions of our study, absence of heparin from the assay did not reduce the activity in the supernatant fractions in Expt. A and C (Table 1) by more than 20%.

Clearing factor lipase is normally distinguished from other lipases by its inhibition by 0.5 M sodium chloride, and by its inability to hydrolyze triglycerides in artificial lipid emulsions, unless these are activated by incubation with serum (3, 18, 19). The lipolytic activity at pH 8.1 of homogenates of fresh heart tissue has these characteristics; thus, in a typical experiment the lipolytic activity of an homogenate (20 mg of tissue/ml of 0.025 N NH_3–NH_4Cl at pH 8.1) prepared from the heart muscle of rats on the normal diet fell from 128 to 4 units/g of tissue (fresh weight) when assayed in the presence of 0.5 M NaCl (95% inhibition). Its activity with the triglyceride emulsion (Intralipid) as substrate was only 3 units/g of fresh weight, but this rose to 102 units when a mixture of serum and emulsion was used. Similar results have been obtained with homogenates prepared from hearts taken from rats starved for 24 hr.

Homogenates of Defatted Tissue. Korn (18) prepared defatted powders from heart muscle by homogenizing the fresh tissue in acetone at 4°C. Because heart muscle in acetone is not easy to disrupt adequately by conventional homogenization techniques, it was found more convenient to homogenize the tissue first in a small volume of an aqueous medium, and then to add the resulting homogenate to a large volume of acetone at 4°C.

Preliminary experiments with this technique showed that the enzyme activity of the defatted powder was higher when the initial homogenization of the fresh tissue was carried out in serum than when it was carried out in water, in 0.025 N NH–NH_4Cl at pH 8.1, or in 5% (w/v) albumin in either water or buffer. The activity was also significantly higher than that of preparations made by Korn's original technique. The results of typical experiments are shown in Table 3. The higher activity of the hearts from starved rats is considered below.

TABLE 3 THE CLEARING FACTOR LIPASE ACTIVITY OF ACETONE–ETHER POWDERS OF HEART MUSCLE PREPARED IN THE PRESENCE AND ABSENCE OF SERUM

Homogenizing Medium for Fresh Tissue	Fed Rats	Rats Starved for 24 hr
	*units of lipase activity/g of tissue**	
Acetone	126	260
Ammonia buffer at pH 8.1	119	267
Albumin (5%, w/v) in water at pH 8.1	129	275
Serum	198	320

Hearts from three rats on the normal diet and from three rats which had been starved for 24 hr, were coarsely minced. Portions of each mince were homogenized in acetone or in the aqueous media described (30 mg of tissue per ml). Acetone–ether powders were then prepared, and duplicate portions of homogenates of the powders were assayed for clearing factor lipase as described in the Methods. The results are the means of the duplicate assays.

* Fresh weight.

The higher activity when serum was used for the initial homogenization could be due to an activating effect on the enzyme assay of serum components in the defatted powder. Powders were, therefore, prepared from a homogenate of the fresh tissue made in 0.025 N NH_3–NH_4Cl (pH 8.1) and, for the assay of the enzyme, portions of the powder were homogenized either in 0.025 N NH_3–NH_4Cl in the usual way (see Methods and Materials), or in serum. No significant differences in activity were found.

The lipolytic activity of homogenates of acetone–ether powders has similar characteristics to that of homogenates of the fresh tissue. Thus, in a typical experiment with homogenates of powders prepared from fresh tissue homogenates in serum, enzyme activity dropped from 288 to less than 5 units/g of tissue (fresh weight) when assayed in the presence of 0.5 M NaCl. With Intralipid the activity was 14 units/g of tissue (fresh weight); whereas, with an Intralipid–serum mixture as substrate, the activity rose to 270 units/g. Similar results have been obtained with powders prepared from fresh tissue homogenates in 5% albumin (w/v) in 0.025 N NH_3–NH_4Cl at pH 8.1.

Though a considerable proportion of the enzyme in homogenates of acetone-ether powders is associated with the tissue residue fraction (4), most is brought into solution when it is assayed in the presence of heparin at 37°C. In experiments similar to those described in Table 2, but carried out with homogenates of acetone–ether powders (15 mg of powder/ml) that had been prepared from fresh tissue homogenates in either serum or in 5% (w/v) albumin in 0.025 N NH_3–NH_4Cl at pH 8.1, only 46% and 34%, respectively, of the total enzyme activity was in solution in the homogenates. However, after 5 min at 37°C, these percentages had risen to 85% and 76%, respectively.

Comparison of the Activities of Homogenates of Fresh and of Defatted Heart Tissue. The results of a direct comparison of the clearing factor lipase activities of homogenates of fresh heart tissue in serum with those of homogenates of acetone-ether powders prepared from the fresh tissue homogenates are shown in Expt. A, Table 4. The higher activity of the defatted preparations is not entirely due to the use of serum for the initial homogenization of the fresh tissue. Thus, the results of Expt. B show that the activity of homogenates of the fresh tissue in 5% albumin (w/v) in 0.025 N NH_3–NH_4Cl (pH 8.1) is also significantly lower than that of homogenates of the corresponding defatted preparations. The difference between Expt. A and B in the activities of the homogenates of the defatted preparations can be attributed to the presence of serum during the powder preparation.

The effect of acetone–ether treatment is apparently restricted to enzyme associated with the tissue residue. Table 5 shows the results of an experiment in which a homogenate of fresh heart tissue was prepared in serum which contained heparin. Most of the enzyme activity of this homogenate was already in solution (Table 1), and the amount associated with the residue fraction was further reduced by extracting it several times with the heparin–serum mixture. Acetone–ether powders were prepared from a portion of the final residue and from a sample of the enzyme in solution. The activity associated with the residue fraction was markedly increased by powder preparation while the activity of the enzyme in solution was not affected. Similar results have been obtained in other experiments in which the homogenates of the fresh tissue were prepared in serum alone so that the activity associated with the residue was not reduced to such a low level before the powders were made (Table 1).

These findings suggest that some clearing factor lipase is present in a homogenate of fresh heart tissue in an inactive form. Consistent with this suggestion, are the results of the experiment described in Table 6. A residue fraction of low activity was separated from a fresh tissue homogenate and treated with deoxycholate (1%). Under these conditions, clearing factor lipase which had not been measurable beforehand, was released from the tissue residue, and the resulting activity was similar to that observed after acetone–ether treatment (Table 5). Such enzyme activity was inhibited by more than 95% in

TABLE 4 A COMPARISON OF THE CLEARING FACTOR LIPASE ACTIVITIES OF HOMOGENATES OF FRESH AND OF DEFATTED HEART TISSUE

Expt.	Homogenizing Medium for Fresh Tissue	Homogenates of Fresh Tissue	Homogenates of Acetone-Ether Powders	Difference	Significance of Difference (P)
		*units of lipase activity/g of tissue**			
A	Serum	100 ± 21	223 ± 28	123 ± 15	<0.001
B	Albumin in ammonia buffer (pH 8.1)	91 ± 14	137 ± 14	46 ± 7	<0.001

In Expt. A hearts from 10 rats on the normal diet were used. After mincing, a portion of each heart was homogenized in serum (30 mg/ml). From each homogenate, samples were taken for the direct assay of clearing factor lipase and for the preparation of acetone–ether powders as described in Methods. Duplicate portions of each powder were homogenized and also assayed. Expt. B was as Expt. A, except that six hearts were used and the fresh tissue was homogenized in 5% (w/v) albumin in 0.025 N NH_3–NH_4Cl at pH 8.1. Values are expressed as the means ±SD.

* Fresh weight.

TABLE 5 THE EFFECT OF ACETONE–ETHER TREATMENT ON THE CLEARING FACTOR LIPASE ACTIVITY OF FRACTIONS OF HOMOGENATES OF FRESH HEART TISSUE

Activity Measured	Supernatant Fraction from Fresh Tissue Homogenate	Residue Fraction from Fresh Tissue Homogenate
	*units of lipase activity/g of tissue**	
Directly	110	7
After acetone–ether powder preparation	113	91

Hearts were taken from five rats on the normal diet and were homogenized in serum (30 mg of tissue per ml) containing heparin (1 IU/ml). After storage at 0°C for 30 min, a portion of the homogenate was centrifuged. The supernatant solution was recovered, and the residue was redispersed in the homogenizing medium to a concentration equivalent to that in the original homogenate. The procedure was repeated four times. Portions of the first supernatant solution and of the final redispersed residue were assayed directly for clearing factor lipase, while other portions were used for the preparation of acetone–ether powders as described in Methods. Portions of homogenates of the powders were also assayed. The results are expressed as the mean activities of assays carried out in duplicate.

* Fresh weight.

TABLE 6 THE EFFECT OF DEOXYCHOLATE ON THE CLEARING FACTOR LIPASE ACTIVITY OF THE RESIDUE FRACTIONS OF HOMOGENATES OF FRESH HEART TISSUE

Treatment	
	*units of lipase activity/g of tissue**
None	2 ± 1
Deoxycholate (1%, w/v)	75 ± 7

Hearts from five rats on the normal diet were homogenized separately in serum (30 mg/ml) containing heparin (1 IU/ml). Each homogenate was kept for 5 min at 37°C and then centrifuged. The tissue residues were redispersed in the original volumes of serum + heparin, and the incubation and centrifugation was repeated. The residues were then dispersed in the same volume of 0.025 N NH_3–NH_4Cl (pH 8.1) containing heparin (1 IU/ml), and portions (5.4 ml) were distributed into tubes containing 0.6 ml of either sodium deoxycholate (10%, w/v) or water. After 10 min at 0°C, centrifugation was repeated. The supernatant solutions were recovered and, after overnight dialysis (4°C) against distilled water, clearing factor lipase was assayed in each sample. Values are expressed as the means ± SD.

* Fresh weight.

the presence of 0.5 M NaCl. Treatment of the residue fraction with Triton X-100 (0.1%) also released enzyme activity, but the measured level was only about 60% of that found with deoxycholate.

The Effect of Fasting on the Activity of Clearing Factor Lipase in the Rat Heart

Homogenates of Fresh Tissue. Hearts were removed between 8 and 9:30 a.m. from rats on the normal diet (hereafter described as fed animals), and from rats which had been starved for various time periods commencing at 8 a.m. on the first day of the experiment. Clearing factor lipase was assayed in homogenates of the fresh tissue, and the results are shown in Fig. 1. Enzyme activity rose progressively during the first few hours of fasting and reached a maximum value after 10–24 hr. Thereafter, with continued fasting, there was a slow decline and after 72 hr, the activity was not significantly higher than that in fed animals.

To verify that the increase in activity during the first few hours was due to fasting per se, experiments were carried out with rats starved from 8 a.m. and then force-fed at noon and at 5 p.m. with either water, glucose, olive oil, or a mixture of glucose, olive oil, and casein hydrolyzate. The results of a typical experiment are in Table 7. They show that glucose alone, or in combination with olive oil and casein hydrolyzate, prevents the rise in enzyme activity which occurs in animals given water. The caloric equivalent of glucose fed in the form of olive oil, on the other hand, is entirely without effect.

Homogenates of Defatted Tissue. The effect of fasting on the clearing factor lipase activity of heart acetone–ether powders is shown in Table 8. The activities of the homogenates of the fresh tissue in serum which were used in the preparation of the powders are also shown for comparison. Activity of the enzyme in acetone–ether powders, like that in the fresh tissue preparations, rises significantly ($P < 0.01$) on fasting, reaches a peak after 8–24 hr, and then declines. Although the activities of the defatted preparations are higher than those of the fresh tissue homogenates throughout the period studied, the differences between the activities of the two preparations decrease with increasing time of fasting and are no longer significant ($P > 0.05$) in the 48 hr starved group of animals.

TABLE 7 THE EFFECT OF FORCE-FEEDING DIFFERENT NUTRIENTS ON THE CLEARING FACTOR LIPASE ACTIVITY OF THE RAT HEART

Group	
	*units of lipase activity/g of tissue**
Water	264 (12)
Olive oil	283 (4)
Glucose	98 (4)
Glucose + casein + olive oil	81 (4)

Food was removed at 8 a.m. from the cages of rats maintained on the normal diet. At noon and at 5 p.m., 4 ml of water, 1 ml of olive oil, 4 ml of glucose solution (60%, w/v), or 4 ml of a solution of glucose (60%, w/v), casein hydrolysate (24%, w/v), and olive oil (5%, w/v) were administered to groups of rats by stomach intubation under ether anesthesia. The animals were killed between 7 and 8 p.m., and the hearts of each group were homogenized together in 0.025 N NH_3–NH_4Cl at pH 8.1 (30 mg/ml). Duplicate samples of each homogenate were assayed for clearing factor lipase as described in the Methods. The number of hearts in each group is given in parentheses.

* Fresh weight.

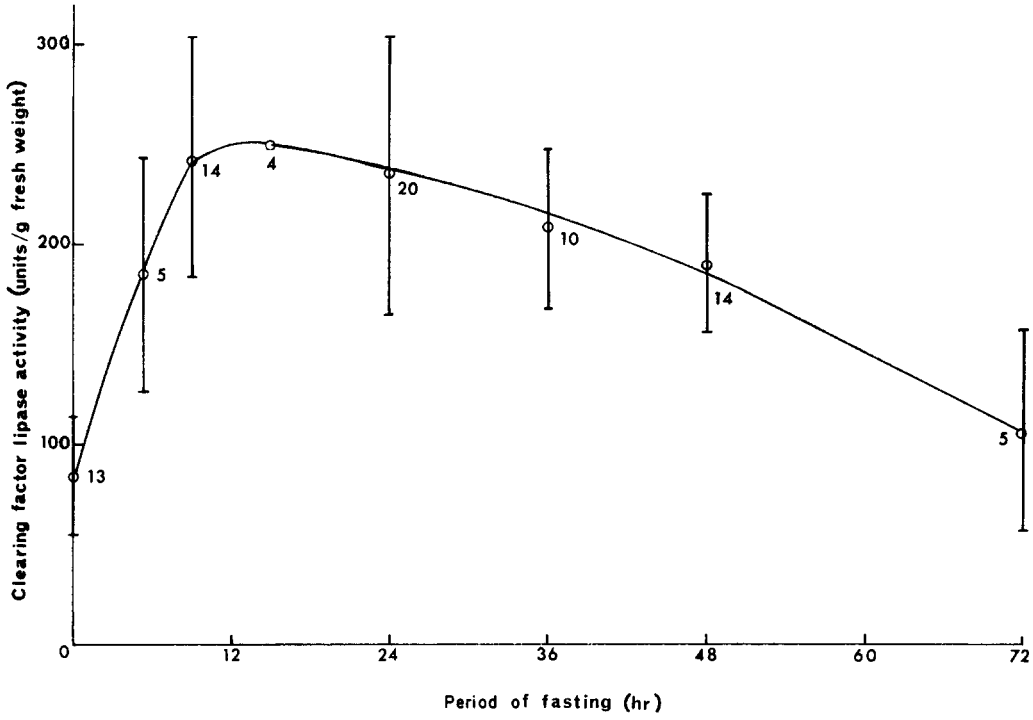

FIG. 1. The effect of fasting on the clearing factor lipase activity of the rat heart. Hearts were taken from rats which were maintained on the normal diet and which were killed between 8 a.m. and 9:30 a.m., and from rats which had been starved for various periods of time starting from 8 a.m. on the first day of the experiment. Clearing factor lipase was assayed in duplicate samples of homogenates of the hearts of each rat in 0.025 N NH_3–NH_4Cl (pH 8.1) (20 mg tissue/ml) as described in Methods. The mean activities at each time interval are recorded. The vertical bars represent SD, and the number of animals in each group is shown in parentheses. All the values for starved animals are significantly greater than those for fed animals ($P < 0.001$), except for the value for animals starved for 72 hr. The values for animals starved for 36 hr or longer are significantly lower than those for animals starved for 10 hr ($P < 0.05$).

The Effect of Fasting on the Activity of Clearing Factor Lipase in Rat Diaphragm

In view of the effects of fasting on the clearing factor lipase activity of the rat heart, similar studies were carried out on diaphragm muscle. Because of the toughness of the muscle fibres it was difficult to obtain satisfactory homogenates of the fresh tissue, and the enzyme was assayed in homogenates of acetone–ether powders prepared from suspensions of the fresh tissue in serum. The results in Table 9 show that an increase in activity occurs on fasting for 8 or 24 hr. Expressed as a percentage of the activity in the muscle of the fed animals, the increases are similar to those found in the rat heart.

DISCUSSION

Assay of Clearing Factor Lipase in Fresh and Defatted Preparations of Rat Heart Muscle

The results of the present study emphasize the importance of measuring the activity of tissue clearing factor lipase under conditions where most of the enzyme is in solution and at its optimum pH between 8.0 and 8.5. Other lipases which act on triglycerides and have a pH optimum between 6.5 and 7.5, appear to be present in heart tissue (7, 20), and these could account for a substantial proportion of the lipolytic activities of homogenates of fresh heart tissue assayed at pH 7.4 (5, 6, 8, 11, 12). They do not appear, however, to contribute significantly to FFA release under the present conditions of assay at pH 8.1 since this release is inhibited almost completely by 0.5 M NaCl, and no activity is exhibited with the triglyceride emulsion, Intralipid, when serum is absent from the assay medium.

Homogenates of fresh tissue have also been shown to contain a monoglyceride lipase (21). By analogy with the monoglyceride lipase of adipose tissue (22), this enzyme may also be expected to be present in homogenates of acetone-ether powders of the heart. However, the monoglyceride lipase also has optimum activity near pH 7 and is active in the presence of 0.5 M NaCl (21). On this basis, it seems unlikely that it will substantially affect measurements of clearing factor lipase activity carried out under the conditions of assay described here.

A measure of the activity of clearing factor lipase in the heart can be obtained by assaying the enzyme which is

TABLE 8 A COMPARISON OF THE CLEARING FACTOR LIPASE ACTIVITIES OF HOMOGENATES OF FRESH AND OF DEFATTED HEART TISSUE IN RATS STARVED FOR VARIOUS PERIODS OF TIME

Period of Fasting	No. of Hearts	Homogenates of Fresh Tissue	Homogenates of Acetone-Ether Powders
hr		*units of lipase activity/g of tissue**	
0	8	93 ± 21	210 ± 25
8	8	210 ± 44	301 ± 57
24	10	253 ± 44	307 ± 48
48	8	141 ± 40	179 ± 38

Hearts were removed from rats, which were maintained on the normal diet and which were killed between 8 a.m. and 9 a.m., and from rats which had been starved for various periods of time from 8 a.m. on the first day of the experiment. Each heart was minced, and a portion of the mince was homogenized in serum (30 mg/ml). From each homogenate, samples were taken for the direct assay of clearing factor lipase and for the preparation of acetone–ether powders as described in Methods. Duplicate portions of each powder were homogenized and also assayed. The values quoted are the means (±SD) of the assays of the individual hearts.

* Fresh weight.

TABLE 9 THE EFFECT OF FASTING ON THE CLEARING FACTOR LIPASE ACTIVITY OF RAT DIAPHRAGM

Expt.	Fed	Starved	
		8 hr	24 hr
	*units/g of tissue**		
A1	55	78	
A2	53	110	
B	48.8 ± 3.5		72.4 ± 13.9

In Expt. A1 and A2, diaphragms were taken from groups of three rats which were maintained on the normal diet and which were killed between 8 a.m. and 9 a.m., and from groups of three rats that had been starved from 9 a.m. and killed at 5 p.m. After coarsely mincing together the diaphragms in each group, suspensions were prepared in serum (50 mg/ml). From these suspensions, acetone–ether powders were made and assayed for clearing factor lipase activity as described in Methods.

In Expt. B a similar procedure was used, but the rats had been fed the normal diet or had fasted for 24 hr when they were killed between 9 a.m. and 9:30 a.m. There were five rats in each group, and each diaphragm was treated separately. The results are means ± SD. The P value for the significance of the difference between the means is <0.01.

* Fresh weight.

extracted from tissue slices incubated in the presence of heparin (6, 8, 23–25). Though such enzyme activity probably represents only a portion of the total in the tissue (4, 26), it could reflect that which participates in TGFA uptake from blood (26, 27). However, it would again seem advisable to assay the extracted enzyme at a pH between 8.0 and 8.5.

The observation that a part of the clearing factor lipase of the heart can only be measured after treatment of the tissue with acetone and ether, or with deoxycholate, is of considerable interest. An explanation for the finding may lie in the unmasking of enzyme normally not active in the tissue, possibly through the removal of lipid. Further work to study this possibility is in progress.

The Effect of Fasting on the Activity of Clearing Factor Lipase in the Rat Heart

The present findings concerning the effect of fasting on the activity of clearing factor lipase in the heart are in agreement with those reported in our earlier study (4), insofar as the enzyme activity of acetone–ether powder preparations is found to be lower in rats starved for 48 hr than in fed rats (Table 8). However, our earlier work clearly presented a grossly oversimplified picture, and the most notable finding in the present study is the rapid and substantial *increase* in heart clearing factor lipase activity which occurs when rats are starved for periods of only a few hours. Such marked dependence of the activity of the enzyme on nutritional status is worth particular emphasis since the rat is normally a nocturnal feeder. Thus, when rats with free access to food are killed late in the day, the clearing factor lipase activity of their hearts would be expected to be appreciably higher than that of hearts from animals killed several hours earlier. Changes have been reported in the activity of clearing factor lipase in the heart after particular treatments which may themselves have taken several hours or which may independently have resulted in an altered nutritional state (11, 12, 24). The possibility that, in at least some of these cases, the observed changes may have been due to a difference in nutritional status, rather than to the treatment per se, cannot be excluded.

Since fasting causes an increase in the activity of clearing factor lipase in both fresh tissue homogenates and acetone–ether powders, the increase in the activity in the former preparation cannot simply be explained by the unmasking of that proportion of the total tissue enzyme which is normally assayed only in the latter. Nevertheless, it is noteworthy that the activities of the two preparations do approach each other in rats starved for long periods (Table 8), and further studies on the relationships between the enzyme activities of the two preparations are required.

The discrepancies between our present findings and those reported by other workers with regard to the effect of fasting on heart clearing factor lipase activity (see p. 102) remain to be explained. An explanation cannot be found in any assumed differences in the nutritional status of the control "fed" animals used in the different experiments. Thus, had other workers used control animals killed later in the day, the clearing factor lipase activity of the hearts would have been expected to have been higher than those in our fed animals, and hence we should have detected more readily the increase in activity reported by others to occur after long periods of starvation (5, 6, 8). The differences in the findings may be explained

rather by the different conditions of assay of the enzyme since other workers carried out assays at pH 7.4 under conditions where lipases other than clearing factor lipase were probably active (*vide supra*). Indeed the results in Fig. 3 of Hollenberg's study (6) suggest that in his experiments only the lipolytic activity measured at pH 7.4 in the absence of serum was increased in the hearts of rats fasted for 3 days. Lipolytic activity measured in the presence of serum, which probably reflects clearing factor lipase activity (3, 18), was not increased.

The rapid rise in the clearing factor lipase activity of the heart during the first few hours of fasting is of particular interest in relation to the marked decline which occurs in the activity of the enzyme in adipose tissue over the same period (28). If the time-course of the activity increase in skeletal muscle which is reported here is similar to that found for the heart, and if changes in activity of the enzyme in particular tissues reflect alterations in their capacity to take up TGFA from the blood (27), then the findings suggest that the plasma TGFA are directed away from adipose tissue and towards muscle during the early stages of fasting. Although decreases in TGFA uptake by adipose tissue and increases in uptake by skeletal and heart muscle in fasting rats have been reported (29), the periods of fasting were longer than the 10–24 hr during which heart clearing factor lipase activity reached its maximum in the present work.

Dr. J. Borensztajn was a Technical Assistance Trainee supported by the Ministry of Overseas Development when this work was carried out. Dr. D. S. Robinson is a member of the External Staff of the Medical Research Council.

Manuscript received 7 August 1969 and in revised form 20 November 1969; accepted 5 December 1969.

References

1. Korn, E. D. 1955. *J. Biol. Chem.* **215:** 1.
2. Korn, E. D. 1955. *J. Biol. Chem.* **215:** 15.
3. Robinson, D. S. 1963. *Advan. Lipid Res.* **1:** 133.
4. Robinson, D. S., and M. A. Jennings. 1965. *J. Lipid Res.* **6:** 222.
5. Zemplenyi, T., and D. Grafnetter. 1959. *Arch. Int. Pharmacodyn. Ther.* **122:** 57.
6. Hollenberg, C. H. 1960. *J. Clin. Invest.* **39:** 1282.
7. Björntorp, P., and R. H. Furman. 1962. *Amer. J. Physiol.* **203:** 323.
8. Alousi, A. A., and S. Mallov. 1964. *Amer. J. Physiol.* **206:** 603.
9. Grafnetter, D., J. Grafnetterova, E. Grossi, and P. Morganti. 1965. *Med. Pharmacol. Exp.* **12:** 266.
10. Gartner, S. L., and G. V. Vahouny. 1966. *Amer. J. Physiol.* **211:** 1063.
11. Mallov, S., and A. A. Alousi. 1967. *Amer. J. Physiol.* **212:** 1158.
12. Mallov, S., and F. Cerra. 1967. *J. Pharmacol. Exp. Ther.* **156:** 426.
13. Salaman, M. R., and D. S. Robinson. 1966. *Biochem. J.* **99:** 640.
14. Fisher, R. A., and F. Yates. 1957. Statistical Tables for Biological, Agricultural and Medical Research. Oliver & Boyd Ltd., Edinburgh, Scotland. 57.
15. Dole, V. P., and H. Meinertz. 1960. *J. Biol. Chem.* **235:** 2595.
16. Kelley, T. F. 1965. *Anal. Chem.* **37:** 1078.
17. Iselin, B., and W. Schuler. 1957. *Helv. Physiol. Pharmacol. Acta.* **15:** 14.
18. Korn, E. D. 1959. *Methods Biochem. Anal.* **7:** 145.
19. Boberg, J., and L. A. Carlson. 1964. *Clin. Chim. Acta.* **10:** 420.
20. Biale, Y., E. Gorin, and E. Shafrir. 1968. *Biochim. Biophys. Acta.* **152:** 28.
21. Yamamoto, M., and G. I. Drummond. 1967. *Amer. J. Physiol.* **213:** 1365.
22. Vaughan, M., and D. Steinberg. 1965. *In* Handbook of Physiology. Adipose tissue. A. E. Renold and G. F. Cahill, Jr., editors. American Physiological Society, Washington, D.C. 239–251.
23. Dury, A. 1961. *Proc. Soc. Exp. Biol. Med.* **107:** 299.
24. Kessler, J. I. 1963. *J. Clin. Invest.* **42:** 362.
25. Nikkilä, E. A., P. Torsti, and O. Penttilä. 1965. *Life Sci.* **4:** 27.
26. Borensztajn, J., and D. S. Robinson. 1970. *J. Lipid Res.* **11:** 111.
27. Robinson, D. S. 1967. *In* The Fate of Dietary Lipids. G. Cowgill and L. W. Kinsell, editors. Public Health Service Publication No. 1742. U.S. Government Printing Office. 166–188.
28. Wing, D. R., and D. S. Robinson. 1968. *Biochem. J.* **106:** 667.
29. Bragdon, J. H., and R. S. Gordon, Jr. 1958. *J. Clin. Invest.* **37:** 574.