Effects of cold exposure on heart clearing factor lipase and triglyceride utilization in the rat

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Abstract The clearing factor lipase activity of the rat heart was measured in animals kept at 4°C for several hours and was compared with that in control animals kept at 25°C. The total activity of the enzyme in the heart increased markedly on exposure to the low temperature, whether the animals were in a fed or a fasted state. The activities of both the heparin-releasable and the heparin-nonreleasable enzyme fractions were usually raised. However, only increases in the former could be correlated satisfactorily with corresponding increases in the capacity of the heart to utilize chylomicron triglyceride fatty acids perfused through it.

Cold exposure also raised the plasma clearing factor lipase activity and reduced the plasma triglyceride concentration. These changes may have been due, at least in part, to the alterations in the activity of the tissue enzyme.

Supplementary key words lipoprotein lipase · chylomicrons

Important evidence that the enzyme clearing factor lipase (lipoprotein lipase) functions to remove triglyceride fatty acids (TGFA) from the blood derives from studies in which changes in the activity of the enzyme in particular tissues have been related to changes in the uptake of TGFA by these tissues. Particularly good correlations exist with respect to the alterations in enzyme activity and TGFA uptake by adipose tissue in fasted and refed animals and by the mammary gland during lactation, and, in recent work with the perfused rat heart, some similar relationships have been established (1).

Work with this last system has shown further, however, that TGFA utilization probably involves only a portion of the total enzyme activity of the organ, namely, that which is readily releasable from the intact heart by heparin and which, as other evidence suggests, is probably localized close to the luminal surface of the endothelial cells of the blood capillaries. For example, removal of the heparin-releasable enzyme from the heart reduces its capacity to utilize TGFA to a very low level. Again, when utilization of TGFA by the perfused heart is high, as in animals that have been fasted for 10–24 hr, the portion of the total enzyme that is rapidly releasable by heparin shows the most marked rise in activity (2, 3).

In the present study, further evidence for the importance of the heparin-releasable enzyme in TGFA utilization has been sought using hearts from rats that have been exposed to low temperatures for short periods. Grafnetter et al. (4) have already reported briefly that such exposure increases the total clearing factor lipase activity of the heart. We have investigated how this increase is distributed between the heparin-releasable and heparin-nonreleasable fractions and whether any accompanying increases in TGFA utilization by the perfused organ can be related to the rise in the enzyme activity of either or both of these fractions. A preliminary report of some of the results has already been presented (5).

MATERIALS AND METHODS

Animals

Female rats of the Wistar strain weighing 180–200 g were used. They were maintained on an Oxoid pasteurized breeding diet containing approximately 50% carbohydrate, 20% protein, 5% fat, 25% moisture, and minerals and undigestible matter. The rats were selected several days before each experiment and put into individual cages in the animal house at 25°C. On the day of the experiment, rats that were to be exposed to 4°C were put in a cold room. Further details of the treatment of the animals are given when the experiments are described.

Perfusion Procedure

Rat hearts were perfused at pH 7.4 in a nonrecirculatory system with Krebs-Henseleit bicarbonate buffer solution containing 5% (v/v) serum and either heparin (5 IU/ml) or ^{14}C-labeled chylomicron TGFA. The technique of perfusion and the methods of measurement of $^{14}CO_2$ evolved from the heart and of ^{14}C incorporation into the heart lipids have been described previously (3). The ^{14}C-labeled chylomicrons used in the perfusions were also prepared as described previously (3) except that they were di-

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

luted in the perfusion fluid to a concentration of 0.45 instead of 0.90 μeq of TGFA/ml. Before dilution, they were washed with albumin (3) to reduce the amount of ^{14}C-labeled FFA, as determined by the method of Kelley (6), to between 0.1 and 0.4% of the total lipid radioactivity.

Clearing factor lipase assay

Clearing factor lipase activity is expressed in terms of the quantity of FFA released at 37°C from a chylomicron triglyceride substrate activated by preincubation with serum (2). One unit of activity represents the release of 1 μmole of FFA during incubation for 1 hr, and activities are expressed as units per gram fresh weight of tissue or per milliliter of plasma.

The method of assay of the enzyme in homogenates of fresh tissue and in samples of heart perfusion fluid has been described (2, 3), and a similar method was used for the assay of the enzyme in plasma. The lipolytic activity demonstrable in heart muscle preparations with this assay has the characteristic properties of clearing factor lipase, e.g., it is inhibited by more than 90% in the presence of 0.5 M sodium chloride (2). In the present study, the lipolytic activity demonstrable in plasma with the assay has been shown to be similarly inhibited. Thus, when rats initially in the fed state were fasted at 25°C or 4°C for 10 hr, the lipolytic activity measured in samples of their plasma fell, respectively, from 0.34 to 0.03 and from 1.5 to 0.04 units/ml when assayed in the presence of 0.5 M sodium chloride. Variations in the concentrations of heparin and serum in the assays resulting, for example, from the presence of these components in the perfusate samples did not affect the enzyme activity (2).

Heparin-releasable and heparin-nonreleasable fractions of clearing factor lipase

Previous studies (7) have shown that the release of clearing factor lipase from the perfused rat heart by heparin is a biphasic process in that a rapid and substantial release of enzyme in the first minute of the perfusion is followed by the release of enzyme at a low level of activity for periods of at least 1 hr. At the end of this period, a considerable proportion of the initial activity of the organ still remains associated with it.

In the present experiments in which heparin was perfused through the heart, clearing factor lipase was usually measured in perfusate samples collected during the first minute and during the following 7 min, as well as in the heart at the end of such an 8-min perfusion. In confirmation of the earlier studies, the enzyme activity in the 1–8-min perfusate sample in all these experiments was low, the activity released per minute being less than 5% of that released in the first minute. Moreover, when changes in the clearing factor lipase activity of the heart occurred, the direction of change of activity in the 1–8-min perfusate sample was the same as that of the enzyme in the heart at the end of the perfusion, whereas this was not always the case with respect to the enzyme in the 0–1-min perfusate sample. Thus, in rats kept at 4°C for 10 hr, the activity of the 0–1-min sample was lower than that in control animals kept for the same time at 25°C, whereas the activities of both the 1–8-min sample and the residual heart enzyme were higher than in the control animals.

These observations suggest not only that the enzyme activity released in the first minute should be distinguished from that released at later times but also that the latter is most appropriately related to the residual heart enzyme. For this reason, the activity in the 1–8-min perfusate samples was combined with the activity of the residual heart enzyme in reporting the results of the study in order to simplify the presentation and also to make easier comparisons with the few experiments in which perfusions were carried out for only 1 min for practical reasons. Thus, in the text and tables the term "heparin-releasable enzyme" refers only to enzyme released during the first minute of a heparin perfusion, and the rest of the activity is termed "heparin-nonreleasable." The term "total activity" is used for the sum of the heparin-releasable and heparin-nonreleasable activities as defined above and also, in those experiments in which the hearts were not perfused with heparin at all (see Table 1), for the activity measured directly in homogenates of the organ.

Chemical methods

Plasma TGFA and glucose were determined as described previously (8). A modification of the Dole and Meinertz (9) method was used for the determination of FFA (2) but with thymol blue as the indicator.

Statistical treatment of results

Where at least four observations were made in a single experiment, the results are expressed as means ± SD. The significance of the difference between two such means was tested by applying Behren's modification of Student's t test (10); if P was less than 0.05, the difference was regarded as significant.

RESULTS

Effect of cold exposure on total clearing factor lipase activity of rat heart

When rats are fasted at room temperature (25°C), the total clearing factor lipase activity of the heart increases, reaching a maximum value after about 10 hr that is two to three times greater than that in fed animals (2). The results in Table 1 show that fasting at 4°C increases the extent of this rise and that, after 10 hr, the clearing factor lipase activity is significantly higher than it is in animals fasted for the same period at room temperature.

In the present study, the heart clearing factor lipase activity of rats in the fed state at room temperature was between 60 and 100 units/g fresh wt of tissue. The results in Table 1 also show that, although there is no rise above this activity when rats are kept for 10 hr at room temperature and given glucose during this period, the activity does increase significantly in animals that are given the same amount of glucose but exposed to 4°C.

Rats kept at 4°C expend more energy in maintaining their body temperature than do animals kept at 25°C. For this reason, although differences in nutrient consumption between the groups kept at 4°C and at 25°C were eliminated in the experiments shown in Table 1, the possibility that the increases in heart clearing factor lipase activity at 4°C were nevertheless due to differences in caloric balance cannot be excluded. However, evidence for a specific effect of cold exposure per se on the heart clearing factor lipase activity is provided in the experiments with 48-hr-fasted rats described below.

TABLE 1. Effect of exposure to 4°C for 10 hr on total clearing factor lipase activity of rat heart

	Heart Clearing Factor Lipase Activity	
	Starved	Fed Glucose
	units/g fresh wt of tissue	
Exposure temperature		
25°C	299 ± 46 (10)	91 ± 23 (5)
4°C	370 ± 43 (10)	189 ± 29 (5)
P values (4°C vs. 25°C)	<0.01	<0.001

Food was removed from the cages of four groups of rats at 9 a.m., and two of the groups were put into a cold room at 4°C while the two other groups remained in the experimental room at 25°C. At 12 noon and at 4 p.m., each of the animals in one of the groups at 4°C and in one of the groups at 25°C was given 4 ml of a 60% (w/v) glucose solution in water by stomach intubation while under light ether anesthesia. Between 7 and 8 p.m. the hearts were removed, rinsed, and weighed, and clearing factor lipase was assayed in aqueous homogenates of the heart ventricles. The results are expressed as means ± SD, the number of animals in each group being given in parentheses.

Effect of cold exposure on clearing factor lipase activity releasable from perfused rat heart by heparin

In view of the evidence that TGFA utilization by the rat heart probably involves only that part of the total clearing factor lipase activity of the organ that is readily releasable by heparin (see Introduction), it was of interest to study the effect of exposure of rats to 4°C on this heparin-releasable enzyme activity. The results in Table 2 show that, in rats that are fasted and kept for 10 hr at 4°C, this activity is, in fact, somewhat reduced below the level found in animals fasted at 25°C. Thus, the higher total heart activity found in such animals is wholly due to the increase in the heparin-nonreleasable enzyme. However, in rats kept for 10 hr at 4°C and given glucose during that period, both the heparin-releasable and the heparin-nonreleasable enzyme activities are significantly higher than in animals kept at 25°C and given glucose, though the absolute increase in the heparin-nonreleasable enzyme activity is still considerably greater.

In a further experiment, groups of rats were kept at either 4°C or 25°C and fed 4 ml of a mixture of casein hydrolysate (12 g), glucose (30 g), olive oil (25 ml), and water (50 ml) at 9 a.m. and at noon and were killed at 3 p.m., 4 hr earlier than in the experiment reported in Table 2. The activity of the heparin-releasable enzyme of the heart (40 ± 29 units/g fresh wt of tissue) in the rats kept at 4°C was similar to that shown in Table 2 and was significantly ($P < 0.01$) higher than that in the rats kept at 25°C (6.0 ± 1.6 units/g fresh wt of tissue). The heparin-nonreleasable enzyme activity was also higher ($P < 0.05$) in the rats kept at 4°C than in those kept at 25°C (124 ± 16 vs. 90 ± 7 units/g fresh wt of tissue) al-

TABLE 2. Effect of exposure to 4°C for 10 hr on clearing factor lipase activity releasable from perfused rat heart by heparin

	Heart Clearing Factor Lipase Activity					
	Starved			Fed Glucose		
	Heparin-releasable	Heparin-nonreleasable	Total	Heparin-releasable	Heparin-nonreleasable	Total
	units/g fresh wt of tissue					
Exposure temperature						
25°C	152 ± 18 (5)	173 ± 23 (5)	325 ± 40 (5)	14 ± 11 (10)	73 ± 7 (10)	87 ± 17 (10)
4°C	130 ± 5 (5)	253 ± 29 (5)	383 ± 27 (5)	47 ± 17 (10)	155 ± 42 (10)	202 ± 45 (10)
P values (4°C vs. 25°C)	<0.05	<0.01	<0.05	<0.01	<0.01	<0.001

Food was removed from the cages of four groups of rats at 9 a.m., and two of the groups were put into a cold room at 4°C while the other two groups remained in the experimental room at 25°C. At 12 noon and at 4 p.m., each of the animals in one of the groups at 4°C and in one of the groups at 25°C was given 4 ml of a 60% (w/v) glucose solution in water by stomach intubation while under light ether anesthesia. The rats were killed between 7 and 8 p.m., and their hearts were perfused for 8 min with Krebs-Henseleit bicarbonate buffer (pH 7.4) containing heparin (5 IU/ml) and 5% (v/v) serum. Clearing factor lipase was measured in the 0–1-min and 1–8-min perfusate samples and in aqueous homogenates of the heart ventricles prepared at the end of each perfusion. In the table, the activity in the 0–1-min perfusate sample is referred to as "heparin-releasable," and that in the 1–8-min sample has been combined with that in the heart ventricles and referred to as "heparin-nonreleasable" (see Materials and Methods). The results are expressed as means ± SD, and the number of animals in each group is given in parentheses.

TABLE 3. Effect of exposure to 4°C for 10 hr on heart clearing factor lipase activity in rats fasted for 48 hr

	Heart Clearing Factor Lipase Activity				
	Heparin-releasable		Heparin-nonreleasable		
	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Total
	units/g fresh wt of tissue				
Exposure temperature					
25°C	73 ± 17	65 ± 9	118 ± 19	113 ± 10	183 ± 16
4°C	93 ± 11	112 ± 18	284 ± 34	270 ± 42	380 ± 30
P values (4°C vs. 25°C)	<0.05	<0.001	<0.001	<0.001	<0.001

Groups of six rats were fasted for 48 hr at 25°C and then, at 9 a.m. on the day of the experiment, one of the groups was put into a cold room at 4°C while the other group remained at 25°C. Between 7 and 8 p.m. the rats were killed and their hearts were perfused with heparin as described in the footnote to Table 2. Heparin-releasable and heparin-nonreleasable clearing factor lipase activities were measured and the results are expressed as means ± SD. The total activities represent the means ± SD of the sums of the heparin-releasable and the heparin-nonreleasable values of Expts. 1 and 2.

though, presumably because of the shorter period of exposure at 4°C, it was not as high as after exposure for 10 hr at 4°C (Table 2).

Effect of cold exposure of rats previously fasted for 48 hr on heart clearing factor lipase activity

In rats fasted for 48 hr, the clearing factor lipase activity of the heart changes only slowly upon continued fasting at room temperature (2). It was of interest, therefore, to carry out experiments to see whether cold exposure in such animals would produce a rise in the activity of the enzyme similar to that observed in animals that had not been fasted before their exposure to 4°C. The results of such experiments are given in Table 3. They show that the total activity of the enzyme in the hearts of 48-hr-fasted animals increases markedly during exposure to 4°C for 10 hr. The levels reached at this time are similar to those achieved in the experiments in which rats originally in the fed state were exposed to 4°C without food (see Table 1). Both heparin-releasable and heparin-nonreleasable enzyme activities are significantly raised during the exposure of the 48-hr-fasted animals to 4°C, and the main effect is again on the latter.

TABLE 4. Effect of nonspecific stress on clearing factor lipase activity of rat heart

	Heart Clearing Factor Lipase Activity		
	Heparin-releasable	Heparin-nonreleasable	Total
	units/g fresh wt of tissue		
Treatment			
48 hr fast	74 ± 14	77 ± 18	151 ± 29
50 hr fast without stress	74 ± 11	80 ± 18	154 ± 27
50 hr fast with stress	96 ± 21	114 ± 19	210 ± 24
P values (stressed vs. non-stressed)	<0.02	<0.001	<0.001

Three groups of 10 rats were fasted for 48 hr at 25°C in a quiet room in the animal house. At 8 a.m. on the day of the experiment, two groups were taken to the laboratory. Over the next 30 min, the hearts of the rats in one of these groups were perfused for 1 min with heparin, and heparin-releasable and heparin-nonreleasable clearing factor lipase activities were measured as described in Materials and Methods. The rats in the other group in the laboratory were disturbed at intervals by running a metal rod along the bars of their cage and then, between 10 and 11 a.m., the hearts of these rats were perfused with heparin alternately with the hearts of rats in the group that had remained undisturbed in the animal house. Heparin-releasable and heparin-nonreleasable activities were then measured in the same way. The results are expressed as means ± SD.

Effect of time of cold exposure on clearing factor lipase activity of rat heart

The time course of the increase in the clearing factor lipase activity of the heart on exposure of rats to 4°C was studied in experiments with animals previously fasted for 48 hr at 25°C in order to minimize enzyme activity alterations due solely to changes in nutritional state. The results in Fig. 1 show that the total enzyme activity of the heart rises rapidly during the first 3–4 hr of exposure and then more gradually during the next 6–7 hr. When the

TABLE 5. Effect of exposure to 4°C for 3 hr on heart clearing factor lipase activity in rats fasted for 48 hr

	Heart Clearing Factor Lipase Activity		
	Heparin-releasable	Heparin-nonreleasable	Total
	units/g fresh wt of tissue		
Treatment			
51 hr fast at 25°C	64 ± 20	83 ± 12	147 ± 9
48 hr fast at 25°C + 3 hr fast at 4°C	123 ± 28	144 ± 10	267 ± 32
P values (4°C vs. 25°C)	<0.01	<0.001	<0.001

Two groups of five rats were fasted for 48 hr at 25°C in a quiet room in the animal house. At 8 a.m. on the day of the experiment, one of the groups was put into a cold room at 4°C while the other group was left undisturbed at 25°C. Between 11 and 11:45 a.m. the rats were killed and their hearts were perfused for 1 min with heparin, and heparin-releasable and heparin-nonreleasable clearing factor lipase activities were measured as described in Materials and Methods. The results are expressed as means ± SD.

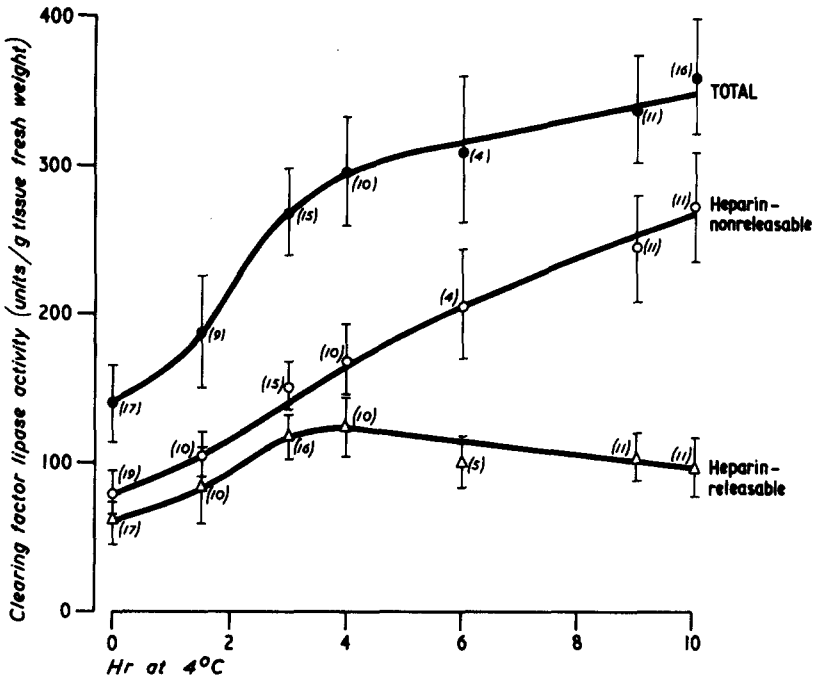

Fig. 1. Effect of time of exposure to 4°C on clearing factor lipase activity of rat heart. Three separate experiments were carried out, and the combined results are shown. In each experiment, rats were fasted for 48 hr and, at 9 a.m. on the day of the experiment, put into a cold room at 4°C. At the times indicated, the rats were killed and their hearts were perfused with heparin as described in the footnote to Table 2. Heparin-releasable and heparin-nonreleasable clearing factor lipase activities were measured, and the total enzyme activities shown (●) represent the sums of these heparin-releasable (Δ) and heparin-nonreleasable (○) activities as measured in each heart. The vertical bars indicate the SD of the measurements at each time interval, and the number of measurements is given in parentheses.

heparin-releasable and heparin-nonreleasable enzyme activities are looked at separately it is seen that their responses to cold are dissimilar. The heparin-nonreleasable activity rises at an almost linear rate for at least 10 hr, whereas the heparin-releasable activity reaches a maximum at 4 hr and then slowly declines. This different behavior of the two fractions clearly explains the biphasic pattern of the total activity curve.

In association with these experiments, control studies were carried out with 48-hr-fasted rats that were kept at 25°C. Over the 10-hr period of study, there was a slight rise in the total heart clearing factor lipase activity from 140 ± 26 to 181 ± 27 units/g fresh wt of tissue. This finding was unexpected in view of earlier work (2) indicating that the total enzyme activity of the heart in rats fasted for 48 hr at 25°C gradually falls with continued fasting. Since the control animals in the present study had been kept in the animal house where they were exposed to a variety of environmental stimuli, it seemed that nonspecific stress might have been the cause of the increase now observed. To test this, the heart clearing factor lipase activity was measured in groups of 48-hr-fasted rats that were kept for 2 hr at 25°C either undisturbed or purposely aroused. The results (Table 4) show that under these conditions there is an increase in the total enzyme activity in the disturbed rats due to increases in both the heparin-releasable and the heparin-nonreleasable enzyme activities. These changes could clearly account for the increase observed in the earlier control experiments.

In the light of this apparent effect of nonspecific stress on both the heparin-releasable and heparin-nonreleasable activities of the heart, a further experiment was carried out in which the activities were measured in 48-hr-fasted animals kept for 3 hr at 4°C or at 25°C in an undisturbed state. The results (Table 5) corroborate those obtained in the earlier experiments (Fig. 1) in showing that the activities of both fractions are increased by such a period of cold exposure. Moreover, comparison of the results with those in Table 3 confirms that the further rise in the total heart activity brought about by more prolonged exposure to 4°C is entirely accounted for by a continuing increase in the heparin-nonreleasable enzyme activity, the heparin-releasable activity showing a slight decline. The enzyme activities in the animals kept at 25°C in this experiment were equivalent to those in unstressed animals (Table 4).

Effect of cold exposure on utilization of triglyceride fatty acids by perfused rat heart

It has been suggested that when ^{14}C-labeled chylomicron TGFA are perfused through the isolated rat heart, the rate of $^{14}CO_2$ production and the extent of incorporation of ^{14}C into the heart lipids is determined by that

TABLE 6. Utilization of ^{14}C-labeled chylomicron TGFA by hearts of rats exposed to 4°C for 10 hr

	TGFA Oxidized					TGFA Incorporated into Heart Lipids	Heart Clearing Factor Lipase
Perfusion period (min)	0–5	5–10	10–15	15–20	Total		
			μeq/g fresh wt of tissue			*μeq/g fresh wt of tissue*	*units/g fresh wt of tissue*
Exposure temperature							
25°C	0.42 ±0.13	0.82 ±0.11	1.00 ±0.11	1.14 ±0.24	3.38 ±0.48	3.00 ±0.40	247 ±45
4°C	0.32 ±0.07	0.62 ±0.14	0.83 ±0.19	0.93 ±0.12	2.70 ±0.42	2.17 ±0.50	338 ±44
P values (4°C vs. 25°C)					<0.05	<0.05	<0.05

Food was removed from the cages of two groups of five rats at 9 a.m.; one group was put into a cold room at 4°C while the other group was left undisturbed at 25°C. Between 7 and 9 p.m. the hearts of the rats were perfused for 2 min with Krebs-Henseleit bicarbonate buffer (pH 7.4) and then for 20 min with buffer containing albumin-washed ^{14}C-labeled chylomicrons (0.45 μeq of TGFA/ml). The perfusates during the 20-min perfusion period were collected over 5-min intervals, and their $^{14}CO_2$ content was determined. At the end of each perfusion, the hearts were perfused again for 2 min with the buffer alone. Portions of the heart ventricles were then taken for measurement of ^{14}C incorporated into the tissue lipids and of clearing factor lipase. Results are expressed as means ± SD.

fraction of the total clearing factor lipase activity that is rapidly releasable from the organ by heparin (3). The finding that exposure of rats at 4°C alters both the heparin-releasable and the heparin-nonreleasable enzyme activities in the heart, and that the direction and extent of the changes in the activities of these fractions depend on the conditions of exposure, has allowed experiments to be carried out to test this hypothesis further.

Table 6 shows the results of an experiment in which ^{14}C-labeled chylomicron TGFA were perfused through hearts taken from rats that had been fasted for 10 hr at either 4°C or 25°C. Though at 4°C under these conditions both the total and the heparin-nonreleasable clearing factor lipase activities of the heart were increased above the level at 25°C, the heparin-releasable enzyme activity was somewhat reduced (Tables 1 and 2). The finding that both oxidation and incorporation of the perfused radioactive lipid were significantly lower ($P < 0.05$) in the hearts of animals kept at 4°C is, therefore, consistent with the view that it is the activity of the heparin-releasable enzyme that primarily determines TGFA utilization by the heart.[1]

A further test of the hypothesis was provided by the results of experiments carried out with 48-hr-fasted rats exposed at 4°C or 25°C for either 3 hr or 8 hr (Table 7). After 3 hr both the heparin-releasable and the heparin-nonreleasable enzyme activities were higher in the hearts from animals kept at 4°C than in those from animals kept at 25°C (Table 5). The finding that both oxidation and incorporation of perfused ^{14}C-labeled chylomicron TGFA were also significantly higher ($P < 0.02$) at this time in the hearts from animals kept at 4°C was not surprising, therefore. However, between 3 hr and 8 hr, while the total and the heparin-nonreleasable enzyme activities continued to increase in the hearts of the animals at 4°C, the heparin-releasable enzyme activity fell (Fig. 1). Therefore, the finding that both oxidation and incorporation of perfused ^{14}C-labeled chylomicron TGFA were also lower after 8 hr than after 3 hr at 4°C, though not significantly so, again suggests that it is the heparin-releasable enzyme that determines TGFA utilization.

Although the declines in oxidation and incorporation with increasing time of exposure to 4°C were not significant in the experiment reported in Table 7, they have always been observed and in some cases have reached a significant level. Thus, in a similar experiment, groups of four rats were fasted for 48 hr at 25°C and then exposed to 4°C for 0, 3, or 8 hr. Oxidation of ^{14}C-labeled chylomicron TGFA during perfusions for 20 min was equivalent to 1.45 ± 0.16 μeq of TGFA/g fresh wt of tissue at 0 hr, and, after 3 hr and 8 hr of cold exposure, the values were 2.31 ± 0.28 and 1.66 ± 0.14 μeq of TGFA/g fresh wt of tissue, respectively (P for 3 hr vs. 8 hr is < 0.01).

Release of clearing factor lipase from rat hearts perfused with chylomicrons

A general finding in the present study has been that the clearing factor lipase activity is lower in hearts perfused with chylomicrons for 20 min at 37°C than in hearts that have not been perfused. This is evident, for example, from a comparison of the results in Tables 1 and 6. Such a difference could be due to inactivation of the enzyme during the perfusions at 37°C. However, an additional possibility is that enzyme is released from the heart by chylomicrons as well as by heparin (11, 12). That such release does

[1] Though the patterns of $^{14}CO_2$ production in this experiment were as described previously (3), the absolute rate of oxidation of the chylomicron lipid by the hearts of the animals kept at 25°C was somewhat higher. This may be accounted for by the lower concentration of lipid perfused through the hearts in the present studies (0.45 vs. 0.9 μeq of TGFA/ml of perfusing medium).

TABLE 7. Utilization of ^{14}C-labeled chylomicron TGFA by hearts of rats fasted for 48 hr at 25°C and then exposed to 4°C for 3 or 8 hr

	TGFA Oxidized					TGFA Incorporated into Heart Lipids
Perfusion period (min)	0–5	5–10	10–15	15–20	Total	
			μeq/g fresh wt of tissue			*μeq/g fresh wt of tissue*
Exposure temperature and time						
25°C, 3 hr	0.14 ±0.08	0.36 ±0.10	0.49 ±0.14	0.44 ±0.08	1.43 ±0.31	1.11 ±0.20
25°C, 8 hr	0.12 ±0.01	0.38 ±0.03	0.52 ±0.04	0.53 ±0.07	1.55 ±0.10	1.29 ±0.10
4°C, 3 hr	0.21 ±0.06	0.63 ±0.13	0.80 ±0.13	0.76 ±0.14	2.40 ±0.41	1.89 ±0.39
4°C, 8 hr	0.18 ±0.06	0.55 ±0.12	0.64 ±0.14	0.67 ±0.14	2.04 ±0.40	1.41 ±0.21

Groups of six rats were fasted for 48 hr at 25°C. Between 7 and 8 a.m. on the day of the experiment, two of the groups were put into a cold room at 4°C while two groups were left undisturbed at 25°C. Heart perfusions were carried out as described in the footnote to Table 6 when the rats at 4°C had been exposed for 3 hr or for 8 hr, the hearts of the rats at 4°C being perfused at the same time as the hearts from the rats kept at 25°C. Portions of the ventricles were taken for measurement of ^{14}C incorporated into the tissue lipids, and $^{14}CO_2$ was measured in each 5-min perfusate sample. Results are expressed as means ± SD.

occur was shown in an experiment in which hearts from six rats that had been fasted for 24 hr at 25°C were perfused with a medium containing chylomicrons for 20 min. In this study the total clearing factor lipase activity in the perfusate was found to be equivalent to 38 ± 15 units/g fresh wt of tissue. In a similar experiment using hearts from six rats fasted for 10 hr at 25°C and then exposed to 4°C and fasted for a further 14 hr, the corresponding value was 49 ± 9 units/g fresh wt of tissue. These values are surprisingly high and this release of enzyme, which presumably occurs from the functional site of its activity at the capillary endothelial cell surface, could well explain why the enzyme activities in hearts that have been perfused with chylomicrons are lower than in those that have not been perfused.

Effect of cold exposure on plasma clearing factor lipase activity and on plasma triglyceride, free fatty acid, and glucose concentrations

In conjunction with the above studies, measurements were made of the plasma clearing factor lipase activity and of the plasma triglyceride, FFA, and glucose concentrations. These are shown in Tables 8 and 9.

Exposure of rats to 4°C for 10 hr caused a significant rise in the clearing factor lipase activity of the plasma above the low level found in the plasma of control animals kept at 25°C. This rise was similar whether the rats were fed or fasted at the time of their exposure to 4°C (Table 8). A significant increase ($P < 0.05$) occurred after 4 hr and persisted for at least 10 hr (Table 9).

The plasma triglyceride concentration falls and the plasma FFA concentration rises on exposure of rats that are initially in the fed state to 4°C for 10 hr (Table 8). This is in agreement with the findings of other workers (13–16). A similar fall in triglyceride concentration occurred on exposure of 48-hr-fasted rats to 4°C, but in such animals the FFA concentration was already high and did not increase further.

Plasma glucose concentrations were measured in the present study only in animals that had been fasted for 48 hr before being exposed to 4°C, and, in these, there was a significant ($P < 0.02$) decline in the first 2 hr (Table 9). Since liver glycogen levels are already low in such fasted animals (17, 18), this fall most likely represents increased utilization of glucose.

DISCUSSION

The results of the present study show that, when rats in a variety of different nutritional states are kept at 4°C, the total clearing factor lipase activity of the heart increases to levels considerably higher than those found in control animals kept at 25°C. Increases in the activities of both the heparin-releasable and the heparin-nonreleasable enzyme occur but, in many situations, the most marked increases are in the heparin-nonreleasable activity. In 48-hr-fasted animals, for example, the heparin-nonreleasable enzyme activity increased steadily and substantially throughout a 10-hr period at 4°C (Fig. 1). On the other hand, though there was an early moderate rise in the heparin-releasable activity, this was followed by a decline so that, after 10 hr, all the increase in total activity was due to the change in the nonreleasable enzyme. That this pattern does not apply only to 48-hr-fasted animals is suggested, moreover, by the findings in force-fed rats exposed to 4°C. Here, cold exposure for 6 hr caused a rise in the activity of the heparin-releasable enzyme, but there was no further increase after 10 hr. The heparin-nonreleasable activity, on the

TABLE 8. Effect of exposure to 4°C for 10 hr on clearing factor lipase activity and triglyceride and free fatty acid concentrations of rat plasma

Nutritional State	Treatment	Plasma Clearing Factor Lipase Activity	Plasma Triglyceride Concentration	Plasma FFA Concentration
		units/ml	*mg/ml*	*μmoles/ml*
Fasted 10 hr	10 hr at 25°C	0.50 ± 0.14	0.50 ± 0.17	0.54 ± 0.11
	10 hr at 4°C	1.23 ± 0.32	0.23 ± 0.03	0.90 ± 0.10
P values (4°C vs. 25°C)		<0.001	<0.02	<0.001
Fasted 58 hr	10 hr at 25°C	0.34 ± 0.26	0.60 ± 0.17	0.76 ± 0.11
	10 hr at 4°C	0.75 ± 0.17	0.31 ± 0.06	0.84 ± 0.10
P values (4°C vs. 25°C)		<0.02	<0.02	NS

Four groups of six rats were used. Two groups were fasted for 48 hr and then, at 9 a.m. on the day of the experiment, one of the groups that had been fasted and one of those that had not were put into a cold room at 4°C; the other groups were left undisturbed at 25°C. At this time food was removed from the cages still containing it. Between 7 and 8 p.m. the rats were bled, and clearing factor lipase, triglyceride, and FFA were estimated in the plasma. The results are expressed as means ± SD.

other hand, was not only raised after 6 hr but increased further after 10 hr at 4°C.

Though the division of the clearing factor lipase activity of the heart into heparin-releasable and heparin-nonreleasable fractions has been made here on a somewhat arbitrary basis (see Materials and Methods), the different behavior of the two fractions on cold exposure suggests that a distinction is generally justified. The results of the experiments on the utilization of chylomicron TGFA by the hearts of cold-exposed animals in different nutritional states show that there is a good correlation between such utilization and the activity of the heparin-releasable enzyme but that no such correlation exists between it and either the total or the heparin-nonreleasable activity. This point is emphasized in Table 10, which summarizes the results presented here and also includes those of additional experiments that were carried out in the course of this study. The primary role of the heparin-releasable enzyme in TGFA utilization by the heart seems to have been generally substantiated by the present work, therefore.

TABLE 9. Effect of exposure to 4°C on clearing factor lipase activity and glucose concentration in the plasma of rats fasted for 48 hr

Time of Exposure to 4°C	Plasma Clearing Factor Lipase Activity	Plasma Glucose Concentration
hr	*units/ml*	*mg/100 ml*
0	0.38 ± 0.11	83 ± 19
2	0.38 ± 0.08	59 ± 7
4	0.58 ± 0.16	58 ± 7
10	0.78 ± 0.22	59 ± 7

Four groups of six rats were used. All the groups were fasted for 48 hr and then, at 9 a.m. on the day of the experiment, three of the groups were put into a cold room at 4°C while the other group was left at 25°C. The rats in the group at 25°C were bled at 9:20 a.m., and those in the groups at 4°C were bled at 11 a.m., 1 p.m., and 7 p.m. After separation of the plasma, clearing factor lipase and glucose were estimated. The results are expressed as means ± SD.

No function can yet be definitely ascribed to the heparin-nonreleasable enzyme. Its properties are similar to those of the heparin-releasable enzyme in that it is completely inhibited in the presence of 0.5 M sodium chloride solution and it will not act upon artificial triglyceride emulsions unless these are appropriately activated by preincubation with serum. Thus, it is difficult to envisage a function for it that is completely different from that of the heparin-releasable enzyme, particularly since hydrolysis of the endogenous triglycerides of the heart can probably be attributed to the presence of a quite distinct lipase (1).

One obvious possibility is that the heparin-releasable and heparin-nonreleasable fractions bear a precursor–product relationship to each other similar to that which has been proposed for two fractions of the enzyme that have been described in adipose tissue (19). However, if such a relationship does exist and the heparin-nonreleasable enzyme is the nonfunctional precursor of the heparin-releasable enzyme at the endothelial cell surface, the question arises as to why cold exposure should sometimes cause the heparin-nonreleasable activity to build up after the heparin-releasable activity has reached its peak. One possibility is that the rate of transport of the enzyme to its functional site may be decreased after cold exposure. Alternatively, cold exposure may cause increased release (or leakage) of enzyme from the functional site into the plasma.[2] That such release normally occurs is suggested by several obser-

[2] It must be emphasized that the plasma clearing factor lipase activities found after cold exposure are much lower than those found after the injection of heparin intravenously. Thus, the highest activity found in the present study (1.23 units/ml of plasma) may be compared with activities of around 100 units/ml of plasma after the injection of heparin. It should also be recognized that the heart clearing factor lipase activity is only a very small proportion of that of the total activity of the body tissues so that its contribution to the plasma activity is probably very small. For example, it can be calculated that the contributions of the total adipose tissue and skeletal muscle masses in the rat are likely to be some two orders of magnitude greater than that of the heart.

TABLE 10. Clearing factor lipase activity of rat heart in relation to its utilization of ^{14}C-labeled chylomicron TGFA

State of Animals	Clearing Factor Lipase Activity			TGFA Utilization during a 20-min Perfusion Period	
	Heparin-releasable	Heparin-nonreleasable	Total	Oxidized	Incorporated
	units/g fresh wt of tissue			*μeq/g fresh wt of tissue*	
Fed glucose at 25°C	13	79	92	0.83	0.56
Fed glucose and kept for 10 hr at 4°C	47	158	205	1.16	0.57
Fasted 48 hr at 25°C and then 3 hr at 25°C	64	83	147	1.43	1.11
Fasted 48 hr at 25°C and then 8 hr at 25°C	75	110	185	1.55	1.29
Fasted 48 hr at 25°C and then 8 hr at 4°C	108	230	338	2.04	1.41
Fasted 48 hr at 25°C and then 3 hr at 4°C	123	144	267	2.40	1.89
Fasted 10 hr at 4°C	130	253	383	2.70	2.17
Fasted 10 hr at 25°C	152	173	325	3.38	3.00

This table is compiled from the results given in earlier tables and in Fig. 1 and from those of additional experiments carried out under the same conditions. The results are arranged so that heparin-releasable clearing factor lipase activity increases from the top to the bottom of the table.

vations in the literature (12) and is probably accounted for by an association of the enzyme with its plasma triglyceride substrate. Thus, whereas the main result of this association is envisaged to be sequestration and hydrolysis of the triglyceride at the endothelial cell surfaces, a secondary one is the release of some of the enzyme into the plasma. Such release presumably is the explanation for the appearance of clearing factor lipase in the perfusate in hearts perfused with chylomicrons that was observed in the present study. Moreover, the postulate that release is increased after cold exposure is consistent with the observation that the activity of the enzyme in the plasma of rats exposed to 4°C is higher than in that of rats at 25°C (Table 8) and with the finding that the plasma enzyme activity begins to rise as the heparin-releasable enzyme reaches its peak after cold exposure (Fig. 1 and Table 9).

The changes in heart clearing factor lipase activity that have been previously reported to occur in fasted rats are accompanied by similar changes in the activity of the enzyme in diaphragm, thigh, and rib-cage muscle (2).[3] If similar events occur in the cold-stressed animal, then the present observations may signify that short periods of cold exposure may cause a general rise in the heparin-releasable enzyme activity of muscle and an increase in the extent of TGFA utilization by this tissue. Such a conclusion would be consistent with work reported by Radomski and Orme (20), when the present study was in progress, showing that postheparin plasma clearing factor lipase activity is increased in cold-exposed rats.[4] Moreover, an increased utilization of TGFA by muscle tissue as a whole in rats exposed for short periods to 4°C could be responsible for the fall in the plasma triglyceride concentration that occurs in such animals. Though there is evidence that the rate of triglyceride release from the liver into the plasma is reduced in rats exposed to cold stress for long periods (21), it is not known whether a similar reduction, which could clearly also contribute to a lowering of the plasma triglyceride concentration, occurs in animals exposed for the shorter periods used in the present study.

Increased utilization of TGFA by muscle tissue as a whole could clearly be of advantage in the shivering thermogenesis that occurs in animals exposed to low temperatures for short periods. Although the plasma free fatty acids are known to be an important source of energy in such conditions (22), their distribution to particular tissues is not readily controlled, being a function primarily of the plasma concentration and of the blood flow (23). A specific increase in the distribution of triglyceride to the musculature, directed through an increased clearing factor lipase activity, could, therefore, help to meet the demands for energy in such a situation.

No attempt has been made in the present study to elucidate the mechanisms involved in the changes in heart clearing factor lipase activity brought about by cold exposure. However, in so far as such exposure can clearly reverse the underlying downward trend in the activity of the enzyme in this organ that is observed during long-term fasting, any hormonal influences involved must presumably act in the opposite direction to, and be distinct from, those operating during starvation.

[3] Robinson, D. S. Unpublished experiments.

[4] These authors also reported, in agreement with the present findings, that the total activity of clearing factor lipase in the heart was raised in such animals, as was that in the lung and in brown adipose tissue, though there was a decrease in the white adipose tissue activity. They did not, however, report any studies on the possible effects of cold exposure on food consumption in their animals, and the possibility that their findings may also reflect an effect of cold exposure on nutritional status cannot be excluded.

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