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Clearing-Factor Lipase in Adipose Tissue

DISTINCTION OF DIFFERENT STATES OF THE ENZYME AND THE POSSIBLE ROLE OF THE FAT CELL IN THE MAINTENANCE OF TISSUE ACTIVITY

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1. Incubation of intact epididymal adipose tissue from fed rats at 37° in an albumin solution at pH 7·4 *in vitro* results in a rapid loss of clearing-factor lipase activity until a low activity, stable to prolonged incubation, is attained. The clearing-factor lipase activity of intact tissue from starved rats, which is initially much less than that of tissue from fed rats, is mainly stable to incubation at 37°. 2. Much of the clearing-factor lipase activity of intact epididymal adipose tissue from fed rats is inactivated by collagenase. The enzyme activity of intact tissue from starved rats is not inactivated by collagenase. 3. The clearing-factor lipase activity of fat cells isolated from the epididymal adipose tissue of fed rats is stable to prolonged incubation at 37°. It represents only a small proportion of the total activity of the intact tissue. In starved rats, the isolated fat cells contain a much higher proportion of the activity of the intact tissue. Their activity is also stable at 37°. 4. Incubation of isolated fat cells in a serum-based medium leads to a progressive rise in clearing-factor lipase activity. Actinomycin increases the extent of this rise in activity. No rise in clearing-factor lipase activity occurs when stromal-vascular cells isolated from epididymal adipose tissue are incubated in the medium. 5. The findings indicate that less than 20% of the activity of intact adipose tissue from fed rats is retained when fat cells are isolated from the tissue by collagenase treatment. The activity that is lost could be that which normally functions in the uptake of triglyceride fatty acids by the tissue.

Removal and uptake of triglyceride fatty acids from the bloodstream by the extrahepatic tissues is believed to depend on hydrolysis of the triglycerides by the enzyme clearing-factor lipase (lipoprotein lipase). Clearing-factor lipase is widely distributed in these tissues and there is considerable circumstantial evidence suggesting that it functions at the surface of the endothelial cells lining the blood capillaries (Robinson, 1963, 1968; Schoefl & French, 1968). FFA* released at this site as a result of triglyceride hydrolysis would be expected to leave the blood and readily enter the tissue cells (Fredrickson & Gordon, 1958).

It has been shown that fat cells can be isolated from the epididymal fat bodies of fed rats by incubating the intact tissue with collagenase at 37° (Rodbell, 1964*a*). Clearing-factor lipase was measured in these experiments and it was concluded that most of the enzyme activity of the tissue was associated with the fat cells (Rodbell, 1964*b*). No clearing-factor lipase activity was found to be associated with the adipose-tissue vascular elements.

* Abbreviations: FFA, free fatty acid(s); CRM, complete reconstituted medium.

These findings appear at first sight to be inconsistent with the view that clearing-factor lipase normally acts in association with the capillary endothelial cells. However, it is now known that the clearing-factor lipase activity of intact adipose tissue from fed rats is unstable at 37° (Wing & Robinson, 1968), and Rodbell's (1964*a*,*b*) experiments were not designed to assess the proportion of the original activity of the tissue that was lost during the incubation period. Moreover, Pokrajac, Lossow & Chaikoff (1967) found that collagenase can, under appropriate conditions, inactivate clearing-factor lipase.

In view of these more recent observations, it seemed worth while to reinvestigate the localization of clearing-factor lipase in adipose tissue by Rodbell's (1964*a*,*b*) technique and, in particular, to determine what proportion of the original tissue activity was lost during the isolation of the fat cells. The findings are presented in the present paper.

The clearing-factor lipase activity of rat adipose tissue falls to a very low level on starvation. Salaman & Robinson (1966) showed that this low

activity rises progressively when the intact tissue is incubated in a suitable serum-based medium *in vitro*. The use of Rodbell's (1964*a*,*b*) procedure to isolate fat cells from adipose tissue has allowed us to determine which cell type in the tissue participates in this increase in activity.

MATERIALS AND METHODS

Adipose tissue. Epididymal fat bodies were taken from male albino rats of the Wistar strain, which had been either fed on their normal diet (Oxoid pasteurized breeding diet) or starved overnight for 15hr. The animals weighed 140–160g. in the fed state and 130–150g. after being starved overnight. All the fat bodies were removed between 9 a.m. and 10 a.m. with the animals under light ether anaesthesia.

Isolation of fat cells. Fat cells were prepared by the method described by Rodbell (1964*a*). All glassware with which the cells might come into contact was dipped into a 2% solution of silicone fluid M.S.1107 (Hopkin and Williams Ltd., Chadwell Heath, Essex) in ethyl acetate, allowed to dry in air at room temperature and then heated at 150° for at least 15min. Five to ten fat bodies were disrupted by incubating them in Krebs–Ringer bicarbonate buffer, pH7·4 (Long, 1961), containing 4% (w/v) bovine serum albumin (fraction V; Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex) and 2–5mg. of collagenase (Worthington Biochemical Corp., Freehold, N.J., U.S.A.)/ml., according to its effectiveness in disrupting the tissue. Approx. 1·5ml. of medium was used for each fat body. Glucose (1mg./ml.) was present when tissue from fed rats was used (Rodbell, 1964*a*). The incubation was carried out under O_2+CO_2 (95:5) in stoppered Erlenmeyer flasks (25ml.), which were shaken in a water bath at 37° for about 1hr. The albumin solution was dialysed beforehand overnight at 4° against a large volume of buffer in dialysis tubing that had been purified by prolonged boiling in water, followed by successive soaking in mM-EDTA (disodium salt), in 10mM-HCl and finally in water.

After the collagenase treatment, undispersed pieces of tissue were removed with forceps, and the fat cells and the stromal-vascular elements were separated by centrifugation at 1000*g* for 1min. The layer of cells floating at the top of the centrifuge tube was recovered and washed twice with 10ml. of the incubation medium from which the collagenase had been omitted. In some experiments the stromal-vascular elements that precipitated on centrifugation were also redispersed and washed in a similar way.

Incubation media. The incubation media used in most of the experiments were variations of the one described above for the isolation of the fat cells and are described in the text. In some experiments the serum-based medium CRM (Salaman & Robinson, 1966) was used, with or without the addition of actinomycin (5μg./ml.).

Techniques of incubation. (*a*) Dispersion of cells. When the washed cells were to be dispersed in a single medium they were poured directly into the medium and agitated gently to effect dispersion. When they were to be distributed into more than one medium, they were first dispersed in a small volume of a medium containing only components common to all the subsequent incubation media. After gentle agitation, samples of this suspension were added to the complete incubation media.

(*b*) Sampling of cells and incubation media. This was carried out while the container was gently agitated to ensure homogeneous dispersion. A silicone-treated pipette, the tip aperture of which was wide enough to prevent damage to the cells (>2mm.), was used to take the samples.

Assay of clearing-factor lipase activity. When samples of the incubation medium alone were to be assayed, separation of the medium from the fat cells was effected by centrifugation for 1min. at 1000*g*. The fat cells formed a layer at the surface, and samples of the medium were taken with a silicone-treated Pasteur pipette. The samples were then re-centrifuged and the procedure was repeated.

When the enzyme activity was to be assayed in the cells and medium combined, acetone–ether-dried preparations were made first. Homogeneous samples of the cells and medium were pipetted directly into 200ml. of acetone at 4°. The precipitates were filtered on Buchner funnels and washed with 200ml. of acetone and then 200ml. of diethyl ether at room temperature. They were dried under reduced pressure and kept at 4°. Within 24hr., portions of the preparations were homogenized in 25mM-NH_3, pH8·1, and the enzyme was assayed in samples of the homogenates. The techniques used were described in detail by Salaman & Robinson (1966).

Acetone–ether-dried preparations were also made from intact fat bodies. A similar technique was used but the bodies were first homogenized in the medium in which they were suspended. A glass homogenizer with a Teflon plunger was used. The homogenate was poured into 200ml. of cold acetone and the procedure described above was followed.

Assay of clearing-factor lipase activity in the above preparations involves measurement of the release of FFA from a chylomicron triglyceride substrate. The method used was that described by Salaman & Robinson (1966) except that the assay medium was slightly modified, to have the following composition: 2vol. of aq. 20% (w/v) albumin, pH8·1; 1vol. of chyle–serum mixture (1:1, v/v); 1vol. of 0·7M-tris–HCl buffer, pH8·1 at room temperature; 0·5vol. of heparin (14i.u./ml.). Usually 2·5ml. of the enzyme preparation was added to 4·5ml. of the assay medium at 37°, and the incubation was carried out by shaking in a water bath for 1·5–2·5hr. The rate of FFA release was linear over these times and FFA were assayed in 1ml. samples taken in triplicate at the beginning and end of the incubation, as described by Salaman & Robinson (1966). Tetra-*n*-butylammonium hydroxide (5mM) in methanol was the alkali used in the titration (Kelley, 1965). Enzyme activities are expressed as μmoles of FFA released from the chylomicron triglyceride substrate/hr. of assay.

RESULTS

Loss of clearing-factor lipase activity on incubation of intact epididymal fat bodies

Wing & Robinson (1968) showed that adipose tissue from fed rats contained a high proportion of a form of clearing-factor lipase that was unstable at 37°. They reported a half-life ($t_{\frac{1}{2}}$) of the enzyme activity under their conditions of incubation of 1–1·5hr. The results in Fig. 1 confirm and extend these findings. When epididymal fat bodies from fed rats were incubated at 37° in the albumin

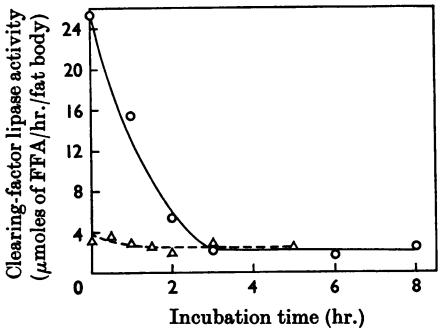

Fig. 1. Loss of clearing-factor lipase activity when epididymal fat bodies are incubated at 37°. The incubation medium was that described in the Materials and Methods section for the isolation of cells, except that no collagenase was present. Glucose (1 mg./ml.) was present in both the experiments. One experiment (○) used tissue from fed rats, and the other (△) tissue from rats that had been starved overnight for 15 hr. In each, 42 fat bodies from 21 rats were incubated in 75 ml. of the medium, and six fat bodies and 10 ml. of medium were removed at intervals. Clearing-factor lipase activity was assayed in homogenates of acetone–ether-dried preparations made from the fat bodies and medium combined.

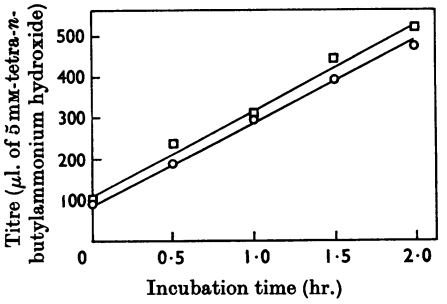

Fig. 2. Lack of effect of collagenase on clearing-factor lipase assay. An acetone–ether-dried preparation of the epididymal fat bodies of a fed rat was homogenized in 25 mM-NH_3, pH 8·1 (see the Materials and Methods section). The clearing-factor lipase activity of the homogenate was assayed in the presence (□) and absence (○) of collagenase (1 mg./ml.). The FFA content of samples of the assay system was measured at intervals and the results are expressed in terms of the titration values.

solution at pH 7·4, there was a rapid decline in clearing-factor lipase activity ($t_{\frac{1}{2}}$ approx. 1 hr.) for about 3 hr. After this time, when some 90% of the original activity of the tissue had been lost, the remaining activity was stable on incubation for a further 5 hr.

Fig. 1 also shows the result of a similar experiment carried out with epididymal fat bodies from rats that had been starved overnight for 15 hr. and in which the initial clearing-factor lipase activity was much lower (Hollenberg, 1959; Cherkes & Gordon, 1959; Pav & Wenkeova, 1960; Salaman & Robinson, 1961). Almost all the activity was stable to prolonged incubation at 37° under such circumstances.

It may be concluded that adipose tissue from fed rats contains two forms of clearing-factor lipase, one unstable and one stable to incubation at 37°, and that the unstable form accounts for a very large proportion of the total activity. In adipose tissue from starved rats, on the other hand, little of the unstable form of the enzyme is present. The activities of the stable form of the enzyme are similar in tissue from fed and from starved animals.

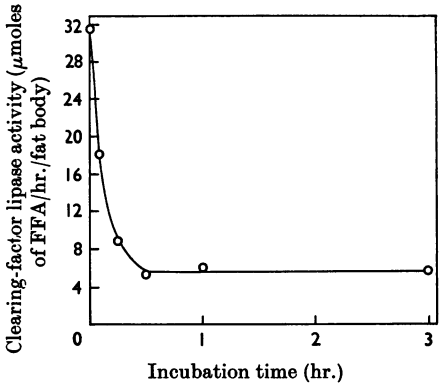

Fig. 3. Loss of clearing-factor lipase activity when epididymal fat bodies are incubated at 37° in the presence of collagenase. Groups of ten epididymal fat bodies from fed rats were incubated in silicone-treated flasks at 37° for specified periods in 15 ml. of the incubation medium used for the isolation of fat cells. The collagenase concentration was 2 mg./ml. The contents of the flasks were then spun for 1 min. at 1000*g* and the infranatant solution was removed. All the remaining tissue components were redispersed and washed twice by suspension and centrifugation in 10 ml. of the incubation medium from which collagenase had been omitted. Finally they were resuspended in 15 ml. of this medium, and clearing-factor lipase activity was measured in homogenates of acetone–ether-dried preparations of the suspensions.

Loss of clearing-factor lipase activity in the presence of collagenase

Inhibition of the enzyme in solution. Part of the tissue clearing-factor lipase may be brought into solution from its location in tissues by heparin (Robinson, 1963, 1968). Pokrajac *et al.* (1967) reported that clearing-factor lipase released from intact parametric fat bodies in this way was

inactivated by incubation with collagenase. This finding was confirmed in the present study. Thus a solution of clearing-factor lipase, extracted with heparin from the epididymal fat bodies of fed rats, was incubated for 10min. at 37° in the albumin medium in the presence or absence of collagenase (1mg./ml.). The enzyme activity in samples of each incubation mixture was assayed directly. The activity in the presence of collagenase was less than 5% of that in the absence of collagenase.

Collagenase added to the assay medium had no effect on the rate of FFA release in these experiments (Fig. 2). Evidence has been obtained suggesting that this lack of inactivation during the assay is due to the presence of serum in the assay medium (V. J. Cunningham & D. S. Robinson, unpublished work).

Inactivation of the enzyme in intact fat bodies. When epididymal fat bodies from fed rats were incubated in the albumin medium at pH 7·4 in the presence of collagenase, a rapid decline in clearing-factor lipase activity occurred (Fig. 3). The rate of decline was much greater than that observed in the absence of collagenase (Fig. 1), approx. 70% of the activity being lost within 15min. At this time, little or no disruption of the tissue had occurred. Subsequently a stable activity was reached. This represented 15–20% of the initial activity, and was similar to that found after prolonged incubation of epididymal fat bodies of fed rats in the absence of collagenase (Fig. 1). No clearing-factor lipase activity was found in the incubation medium.

The activity remaining after incubation in the presence of collagenase varied in different experiments between 2 and 8 μmoles of FFA released/hr./fat body. To compare directly the activity eventually attained in an incubation in the presence of collagenase with that in its absence, a further experiment was carried out by using a paired-fat-body technique. The activity of five fat bodies taken from fed rats and incubated for 4hr. in the presence of collagenase (6·1 μmoles of FFA released/hr./fat body) was not appreciably different from that of five paired fat bodies incubated under the same conditions in the absence of collagenase (7·3 μmoles of FFA released/hr./fat body). Such activity was inhibited by over 90% in the presence of 0·5M-NaCl and was not exerted against artificial triglyceride emulsions unless these had been activated by incubation in the presence of plasma. The enzyme thus possessed the properties of clearing-factor lipase (Robinson, 1963).

It is concluded from these experiments that the unstable, but not the stable, clearing-factor lipase activity in fat bodies from fed animals is inactivated by collagenase.

Inactivation of the enzyme in disrupted isolated fat cells. Clearing-factor lipase activity is associated with intact fat cells, which can be isolated from adipose tissue after its incubation with collagenase (Rodbell, 1964*b*; see below). Isolated fat cells can be disrupted by making an acetone–ether-dried preparation: when a homogenate of such a preparation in 25mM-ammonia was incubated with collagenase for 10min. at 37° at pH 7·4, virtually all the enzyme activity was lost (Table 1).

Table 1. *Inactivation by collagenase of clearing-factor lipase in disrupted isolated fat cells*

Isolated fat cells were prepared as described in the Materials and Methods section, and resuspended in 4% (w/v) albumin in Krebs–Ringer bicarbonate buffer, pH 7·4. Acetone–ether-dried preparations of the suspensions were made and homogenized in 25mM-NH_3, pH 8·1 (Salaman & Robinson, 1966). After adjustment of the pH to 7·4 with M-HCl, samples of each homogenate were incubated, with or without the addition of collagenase (1mg./ml.), for 10min. at 37° and then assayed for clearing-factor lipase activity. The assays were carried out at pH 8·1 in the medium described in the Materials and Methods section.

	Clearing-factor lipase activity (μmoles of FFA released/hr./fat body)	
Expt. no.	Extract incubated without collagenase	Extract incubated with collagenase
1	2·80	0·16
2	5·20	0·30
3	2·24	0·0

Loss of clearing-factor lipase activity during the preparation of isolated fat cells

Evidence has been presented above suggesting that, when fat cells are isolated by incubation of intact adipose tissue of fed rats in a solution containing collagenase at 37° (Rodbell, 1964*a*,*b*), a loss of clearing-factor lipase activity may occur because a considerable proportion of the enzyme is unstable under the incubation conditions, and collagenase inactivates the enzyme. The results in Table 2 show the extent of this loss of clearing-factor lipase activity in our experiments, carried out under the conditions described by Rodbell (1964*a*,*b*). With fat bodies from fed rats, the total activity of the incubation system after 1hr. was only 10–15% of the original. The finding that, apart from the activity in the undispersed tissue, virtually all the residual clearing-factor lipase activity was in the isolated fat cells is in agreement with Rodbell's (1964*a*,*b*) results. But it must clearly be seen in the context of the large overall loss of enzyme activity.

Also shown in Table 2 are the results of similar experiments with epididymal fat bodies from rats that had been starved overnight for 15hr. Again, apart from activity in the undispersed tissue, all the

Table 2. *Clearing-factor lipase activities of the tissue fractions produced during the isolation of fat cells from epididymal fat bodies*

Epididymal fat bodies were taken from fed rats or from rats that had been starved overnight for 15hr. Six fat bodies from six rats in each experiment were incubated in the presence of collagenase and glucose (1mg./ml.) for 1hr. Acetone–ether-dried preparations were made of isolated fat cells, stromal-vascular cells and undispersed tissue after this period, by using techniques described in the Materials and Methods section. An acetone–ether-dried preparation was also made of the six paired intact fat bodies immediately after their removal from the animals. Clearing-factor lipase activity was assayed in homogenates of the preparations. The activities of the fractions as a percentage of the activity of the intact tissue are given in parentheses.

		Clearing-factor lipase activity (μmoles of FFA released/hr./fat body)		
Expt. no.	Source of tissue	Fat cells	Stromal-vascular cells	Undispersed tissue
1	Fed rats	4·9 (13%)	0·2 (0·4%)	0·6 (1·6%)
2	Fed rats	4·6 (9·7%)	0·2 (0·3%)	0·6 (1·3%)
3	Starved rats	4·4 (90%)	0·1 (2%)	0·5 (11%)
4	Starved rats	4·9 (91%)	0·2 (3%)	0·7 (13%)

residual clearing-factor lipase activity was associated with the fat cells. However, as expected from the earlier findings, the percentage loss of activity during the incubation was much less than when fat bodies from fed rats were used. The final activity in fat cells was similar whether tissue from fed or from starved animals was used as the starting material.

Comparison of the effect of incubation in CRM on the clearing-factor lipase activity of isolated fat cells and intact epididymal fat bodies

Increase in the clearing-factor lipase activity of isolated fat cells on incubation in CRM. Salaman & Robinson (1966) showed that the low clearing-factor lipase activity of epididymal fat bodies taken from starved rats increased progressively over a period of several hours when they were incubated in an appropriate serum-based medium, CRM. The results in Table 3 show that a similar increase in activity occurred with fat cells isolated from the fat bodies. Cells isolated from the tissues of either fed or starved animals behaved similarly. It is not possible to compare directly the increases in activity shown here with those observed by Salaman & Robinson (1966) since the yield of cells from the fat bodies is unknown. However, the increases are of a similar order of magnitude.

Because heparin was a constituent of the incubation medium used by Salaman & Robinson (1966), enzyme was extracted from the fat bodies as the incubation progressed. In fact, the time-course of the increase in total enzyme activity was most readily measured by following the increase in the enzyme activity of the medium. A similar situation was found with isolated fat cells (Fig. 4). The increase was rapid for about 4hr. and then slowed down; the activity reached a plateau, as recorded by Salaman & Robinson (1966). Also shown in Fig. 4 are the results of an experiment in which stromal-vascular cells, obtained during the isolation procedure, were also incubated in CRM. No increase in enzyme activity was observed with this tissue fraction.

Table 3. *Increase in total clearing-factor lipase activity when isolated fat cells are incubated in CRM*

In each experiment, isolated fat cells, prepared as described in the Materials and Methods section from the epididymal fat bodies of two rats, were resuspended in 10ml. of the serum-based medium (CRM) described by Salaman & Robinson (1966). Acetone–ether-dried preparations were made from homogeneous samples of cells and medium, taken initially and after incubation for 3·5hr. at 37°. The clearing-factor lipase activities of these preparations were assayed in duplicate, and mean values are quoted.

			Total clearing-factor lipase activity (μmoles of FFA released/hr./fat body)	
Expt. no.	Source of cells	Time of incubation (hr.)	0	3·5
1	Starved rats		5·3	10·9
2	Starved rats		5·0	9·2
3	Fed rats		3·4	7·5

From a comparison of these experiments with those reported in Table 3 it appears that most of the increase in total enzyme activity that occurs when isolated fat cells are incubated in CRM may be accounted for by the increase in enzyme activity in the medium. This has been confirmed in experiments in which enzyme activity in the incubation medium and in the cells was measured separately before and after incubation. Enzyme activity

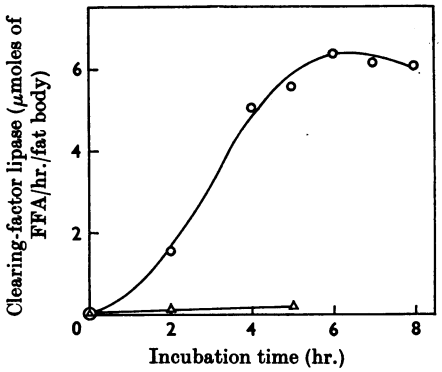

Fig. 4. Increase of clearing-factor lipase activity in the medium when isolated fat cells and stromal-vascular cells are incubated in CRM. Isolated fat cells and stromal-vascular cells were prepared as described in the Materials and Methods section from eight epididymal fat bodies of rats that had been starved overnight for 15hr. The isolated fat cells were resuspended in a final volume of 27ml. of CRM (see Table 3), and incubated at 37°. At intervals, homogeneous samples of cells plus medium were taken, and the medium was assayed directly for clearing-factor lipase activity after the cells had been spun off (○). Stromal-vascular cells from the same preparation were resuspended in 10ml. of CRM and homogeneous samples of this incubation medium were also assayed directly at intervals (△).

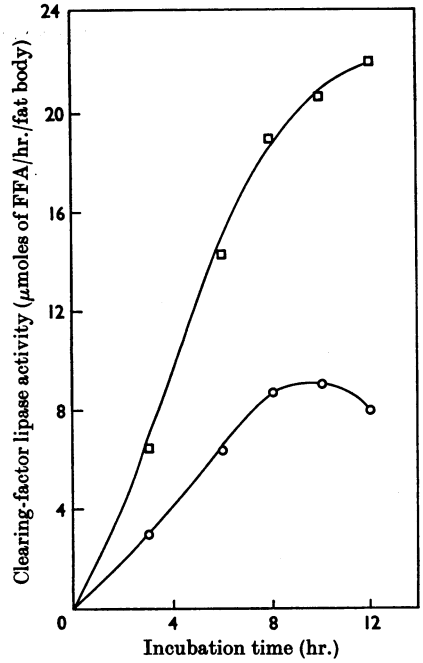

Fig. 5. Increase of clearing-factor lipase activity in the medium when isolated fat cells are incubated in CRM in the presence of actinomycin D. Isolated fat cells were prepared from the epididymal fat bodies of eight rats that had been starved overnight for 15hr. The cells were distributed equally between two flasks, each containing 25ml. of CRM (see Table 3). One flask (□) contained actinomycin D (5μg./ml.) whereas the other (○) did not. During incubation at 37°, homogeneous samples of cells plus medium were taken at intervals and the medium was assayed directly for clearing-factor lipase activity after spinning off the cells.

increased in the medium, but that in the cells did not change significantly. The observation is essentially in agreement with the findings obtained with intact fat bodies (Wing, Salaman & Robinson, 1966).

Increase in the clearing-factor lipase activity of isolated fat cells on incubation in CRM in the presence of actinomycin D. Wing & Robinson (1968) showed that the increase in clearing-factor lipase activity that occurred when epididymal fat bodies from starved rats were incubated in CRM was greatly augmented if actinomycin D was present. A similar effect was observed with isolated-fat-cell preparations (Fig. 5).

DISCUSSION

On the basis of the results of the present study and of the work of Wing & Robinson (1968), it is possible to distinguish at least two states of clearing-factor lipase in rat adipose tissue. In fed animals, the high clearing-factor lipase activity in the tissue appears to be due predominantly to an enzyme activity that is unstable to incubation at 37° and that is inactivated when the intact tissue is treated with collagenase. A small proportion of the total activity is due to an enzyme activity associated with the fat cells of the tissue that is stable to incubation at 37°, and that is only inactivated by collagenase when the fat cells are disrupted by acetone–ether treatment. In starved animals, the activity of the enzyme in the unstable state is markedly decreased, but the activity of the stable enzyme associated with the fat cells is similar to that in tissue from fed animals.

These findings indicate that lack of inactivation by collagenase (and, possibly, enzyme stability) may derive simply from the association of clearing-factor lipase with the intact fat cell. If this is so, then it is reasonable to conclude that the unstable enzyme inactivated by collagenase is at a site in the tissue outside the fat cell. The activity that is lost when intact tissue from fed animals is treated with collagenase certainly could be released from the cells during the incubation of the tissue with collagenase. However, inactivation occurs rapidly before there is any significant physical disruption of the tissue.

The view that clearing-factor lipase is present at two sites in adipose tissue does not, however, exclude the additional possibility that the enzyme may exist in more than one form. Wing & Robinson (1968) distinguished a soluble form of clearing-factor lipase, induced in adipose tissue in the presence of actinomycin D, which differed in its stability at 37° from that which could be extracted from tissue from fed rats. Thus, the ready inactivation by collagenase of the enzyme associated with isolated fat cells, after acetone–ether treatment of the cells, could be due either to release of the enzyme from an inaccessible site in the cells or to the conversion of the enzyme from a form not susceptible to inhibition by collagenase, into a susceptible form.

In the light of the present work, the transformation from the starved to the fed condition in the rat appears to involve an increase in unstable clearing-factor lipase activity in adipose tissue at a site outside the fat cells. The finding that the increases in clearing-factor lipase activity, previously observed with intact tissue from starved animals *in vitro* on incubation in a serum-based medium (Salaman & Robinson, 1966; Wing *et al.* 1966), can be reproduced with isolated fat cells from either fed or starved animals, but not with stromal-vascular cells, suggests nevertheless that the increase in enzyme activity is initiated in the fat cells.

Two observations in this study are not in agreement with earlier work carried out by other investigators on isolated fat cells. Thus Pokrajac *et al.* (1967) found that the clearing-factor lipase activity of isolated cells from starved rats was lower than that of cells from fed rats. This was not the case in the present investigation. However, Pokrajac *et al.* (1967) used rats that had been starved for 72hr. It is possible that, after such a time, the activity of clearing-factor lipase in the fat cells may be decreased below that in tissue from rats starved for 15hr., as in this study. Moreover, Pokrajac *et al.* (1967) measured clearing-factor lipase activity in aqueous homogenates of the fresh tissue, whereas the activity of acetone–ether dried preparations was determined here. Ho, Ho & Meng (1967) reported that the stromal-vascular cells of adipose tissue contain a lipase with some of the characteristics of clearing-factor lipase. Although we were unable to obtain any evidence for this, it should be pointed out that our studies do not indicate precisely where outside the fat cells the unstable enzyme is located.

Finally, the present study seems to dispose of the apparent difficulty created by the initial observation by Rodbell (1964*b*) about the localization of clearing-factor lipase in adipose tissue (see the introduction). The fact that, in the fed animal, more than 80% of the enzyme activity may be due to enzyme outside the fat cells, allows the retention of the original concept of the function of the enzyme in the uptake of triglyceride fatty acids from the blood.

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