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PROPERTIES OF A HORMONE-STIMULATED LIPASE FROM RAT ADIPOSE CELLS*

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Abstract—A well defined, easily reproducible system for the study of hormone-stimulated lipolysis in broken cell preparations from rat adipocytes has been described. Addition of hormone prior to cellular rupture results in a homogenate which liberates free fatty acids (FFA) in a linear fashion for at least 1 hr. The quantity of FFA liberated from nonhormone-treated control homogenates is much smaller but is also linear for at least 1 hr. The activation process occurring after addition of hormone appears to be complete within 1 min. Preincubation of the cells prior to addition of the hormone does not increase the magnitude of the response to the hormone. Broken cell preparations made from both control and hormone-treated cells show a pH optimum in the range of 6·5–7·5, and both preparations show a necessity for the presence of bovine serum albumin in the assay medium. The centrifugation of an activated homogenate at 0° and subsequent recombination of the resulting fractions (fat cake, infranatant and pellet) produced a preparation showing lipolysis. When the infranatant from an untreated homogenate was recombined with the fat cake from a hormone-treated homogenate, hormone-stimulated lipolysis was still seen; the reverse combination showed no such stimulation of lipolytic activity. When the infranatant fluid was removed entirely and replaced with warm (37°) Krebs–Ringer phosphate buffer (KRP), lipolysis was greater in hormone-treated than in control preparations, and the specific activity was increased 5-fold. It is tentatively concluded, therefore, that the "active" lipolytic properties are associated with the fat cake; the infranatant fluid appears to inhibit the lipolytic process.

IT HAS been previously established that catecholamines increase lipolysis in adipose tissue, both *in vivo* and *in vitro*.[1,2] Furthermore, it has been reported that catecholamine-stimulated lipolytic activity can be seen in broken cell preparations from adipose tissue, provided that, in most cases, the hormone is administered prior to cell disruption.[3–7] At the present time, a wide variety of methods *in vitro* has been used to demonstrate hormone-stimulated lipolytic activity; in addition, several different tissue preparations have served as the starting point for such studies, including intact adipose organs (epididymal fat pads), tissue slices and minces, and isolated adipocytes.

Due, in part, to this variety of experimental approaches, many of the reported results have proved difficult to reproduce and the characteristics of catecholamine-stimulated lipolytic activity in adipose tissue are still only imprecisely defined. The first purpose of this investigation, therefore, was to define clearly one method which has been used in studying the effects of catecholamines on lipolysis in adipose tissue. This method has the advantage of being easily reproduced; it involves the study of a broken cell preparation obtained from isolated adipocytes. Furthermore, the characteristics of both basal and hormone-stimulated lipolytic activity within this system have been investigated.

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When homogenates of adipose tissue are centrifuged, the lipolytic activity, both basal and hormone-stimulated, is frequently localized in one portion of the centrifugate. Thus, Strand *et al.*[8] have reported hormone-stimulated lipolytic activity associated with the fat layer of such a centrifugate. Likewise, Hollett[9] has found a similar association of lipolytic activity in the oil layer formed upon centrifugation of such a homogenate at greater force. On the other hand, Rizack[3] has reported similar activity in the infranatant fluid, while Rubenstein *et al.*[5] found lipolysis in all three components of the centrifugate (fat layer, infranatant and pellet).

Since the location of lipolytic activity appeared to depend on the experimental methods employed, a second purpose of this work was to investigate the localization of both basal and catecholamine-stimulated lipolytic activity in this precisely defined system. Such a study is, hopefully, the first step in a more complete characterization of the nature of the hormone-induced lipolytic response in adipose tissue.

METHODS

Preparation of cells. Isolated adipocytes were prepared from the epididymal fat pads of decapitated, exsanguinated, Holtzman rats weighing 150–200 g in accordance with the method of Rodbell,[10] as modified by Lech and Calvert.[11] The isolated fat cells thus prepared and suspended in Krebs–Ringer phosphate (KRP) buffer were divided into two portions, labeled A and B.

Activation step. The B cells were treated with 10^{-5}M norepinephrine (NE) and 10^{-3}M theophylline (theo) (final concentrations). The A cells served both as a control and as a measure of basal lipolysis. The cells were exposed to the above mentioned agents for a period of 1 min. After this exposure period, the cells were homogenized via sonication with a Branson Sonifier Cell Disruptor (microtip setting of 7) for a period of 2·5 sec/ml of cells.

Assay step. The lipolytic activities of both control (A) and hormone-treated (B) homogenates were determined by means of a 1-hr incubation at 37° in a Dubnoff metabolic shaking incubator. The assay medium consisted of: (1) 10 m-moles phosphate buffer, pH 7·0; (2) 4% bovine serum albumin; (3) 0·2 ml homogenate; and (4) glass-distilled water up to a total assay volume of 1·0 ml. In addition, 10^{-5}M NE and 10^{-3}M theo (final concentrations in assay medium) were routinely added to a sample of homogenate A as a check for incomplete homogenization.

The free fatty acids (FFA) produced during the incubation period were extracted and titrated according to the method of Dole and Meinertz.[12] The protein content of the homogenates was determined via the method of Lowry *et al.*[13] using crystalline bovine serum albumin as a standard.

Centrifugation and recombination. Cells were prepared, divided, activated and sonicated as described above. The activity of the homogenates was assayed as a preliminary step. Both homogenates A and B were then centrifuged at 12,000 *g* for 10 min at 0°. After centrifugation, the fat cakes and infranatant fluids were removed, recombined and resonicated. The lipolytic activity of the recombined homogenates was then assayed.

Crossover of infranatants. Both activated and control broken cell preparations were prepared and assayed for lipolytic activity. The homogenates were centrifuged for 10 min at 12,000 *g* at 0°. The fat cakes and infranatant fluids thus formed were removed

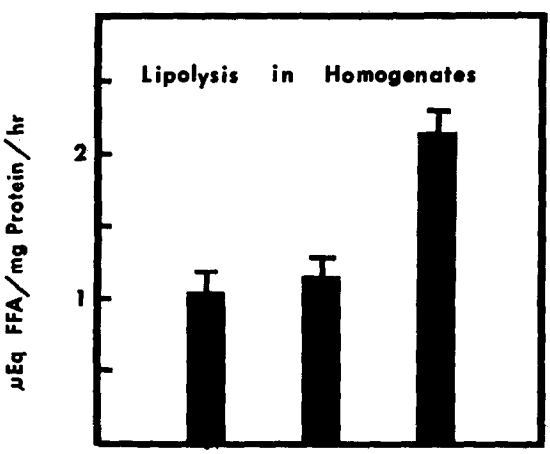
and separated. The fat from A was then recombined with infranatant A or infranatant B and resonicated (2·5 sec/ml of homogenate); likewise, the fat cakes from B were recombined with infranatant A or infranatant B and resonicated. The recombined homogenates were then assayed as described above.

Replacement of infranatants with KRP. Cells were prepared, activated, sonicated and assayed. Both homogenates were centrifugated at 12,000 *g* for 10 min at 0°. The infranatant fluids of both homogenates A and B were then removed, discarded and replaced with an equal volume of KRP buffer. The homogenates were then resonicated and assayed for lipolytic activity as above.

Materials. The materials used in this study were obtained as follows: Bovine Serum Albumin, Fraction V, from Armour Pharmaceutical Company, Chicago, Ill.; Collagenase, *Cl. histolyticum*, from Nutritional Biochemicals Corp., Cleveland, Ohio. All other chemicals employed were of the highest purity commercially available.

RESULTS

Figure 1 shows the results of the activation and assay of hormone-stimulated lipolytic activity in isolated adipocytes using arbitrarily chosen conditions for stimulation and assay. These results are expressed as the change (Δ) in microequivalents

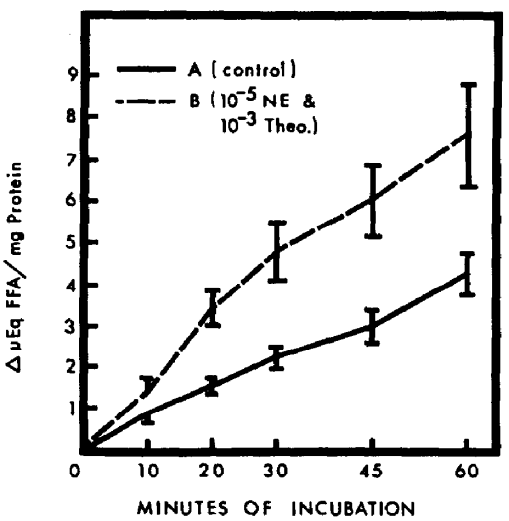


Analysis of Variance	d. f.	F	Sig. Level
A vrs. B	1 & 24	18·18	·01
A + NE vrs. A	1 & 24	0·44	n. s.

FIG. 1. Effects of NE and theo on broken cell preparations made from isolated adipocytes. A represents the lipolytic activity of nonhormone-treated homogenates; B represents the activity of homogenates from cells treated with 10^{-5} NE and 10^{-3} theo prior to cell disruption (see Methods); "A + NE + theo in assay step" represents the lipolytic activity of homogenates A with 10^{-5} NE and 10^{-3} theo present during the assay step. Values given are means of 13 experiments ± S.E.M. Incubation period was 60 min at 37°.

of FFA per milligram of protein per hour (60 min minus 0 time titration). The preparations to which norepinephrine (NE) and theophylline (theo) were added prior to cell disruption (bar B) show significantly greater lipolytic activity than the control preparation (bar A). On the other hand, adipocyte preparations to which NE and theo were added after cell disruption (bar A plus NE and theo in assay step) show no significant difference from control values. The addition of NE and theo to the assay step served as an index of the completeness of homogenization, since the presence of any whole cells would be reflected in an increase in the lipolytic activity of homogenate A.

Figure 2 illustrates the time course of the liberation of FFA over the 1-hr incubation period. Again the difference in lipolytic activity between hormone-treated (curve B) and control (curve A) preparations can be seen ($P < 0{\cdot}01$ at 20 min). In addition, this



Curve	d. f.	F	sig. level	Conclusion:
A	1 & 3	328·50	0·01	Linear
B	1 & 3	72·17	0·01	Linear

FIG. 2. Time course of FFA liberation over 60-min incubation period. NE and theo were added to preparation B prior to cell disruption (see Methods). Points represent the means of five experiments ± S.E.M. Incubation temperature was 37°.

figure shows that FFA production is linear throughout the entire incubation period. The statistics at the bottom of the figure demonstrate that the regression of FFA production on time is linear for 1 hr in both control (A) and hormone-treated (B) preparations. A 1-hr incubation was, therefore, used in all subsequent experiments.

In Fig. 3, three dose–response curves are presented. The solid curve (NE alone) shows a peak response of 3 μequiv./mg protein/hr at 10^{-5}M NE. Both above and below this concentration, FFA liberation was reduced. The dashed curve represents the lipolytic response to theophylline alone. No significant stimulation of lipolytic activity was seen until the theophylline concentration reached 5×10^{-3}M ($P < 0{\cdot}01$).

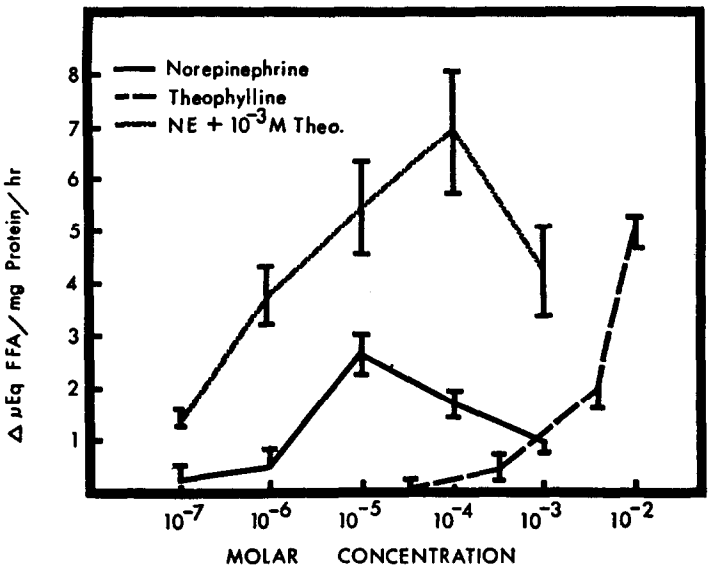


FIG. 3. Dose–response curves of NE, theo, and NE in the presence of 10^{-3} theo. Drugs were administered to adipocytes prior to cell disruption (see activation, Methods). Points represent the means of 5 experiments ± S.E.M. Incubation period was 60 min at 37°. Note that Δ FFA in this figure represents hormone-stimulated (B) minus control (A) values.

From this point on, further increases in theo concentration resulted in greater stimulation of lipolytic activity. The third and diagonally marked curve represents the dose–response curve of NE in the presence of 10^{-3}M theo. This curve shows a peak response of 7 μequiv. FFA/mg protein/hr at 10^{-4}M NE.

Figures 4 and 5 show the results of alterations of the activation step. Figure 4 illustrates the results of varying the length of the preincubation period (the time between the final wash of the cells and the addition of NE and theo). It can be observed that a preincubation period of up to 2 hr does not significantly alter either the basal lipolytic activity (A) or the hormone-stimulated activity (B).

Figure 5 shows the results of varying the duration of exposure of the whole adipocytes to NE and theo (the time period between stimulation and sonication of the cell preparations). It can be seen that maximal hormonal stimulation of lipolytic activity is attained within 1 min of the addition of the hormone to the cell preparation. Exposure of the cells to the hormone for longer than this 1-min period does not result in any greater stimulation of lipolytic activity (as shown by the 2-min and 5-min bars).

The results of two variations in the assay step are presented in Figs. 6 and 7. Figure 6 shows the results of varying the per cent of bovine serum albumin (BSA) in the assay medium. The broken cell preparations from nonstimulated cells (curve A) show maximum lipolytic activity with 2% BSA in the assay medium. Further increases in BSA concentration appear to be slightly inhibitory on nonstimulated lipolytic activity. Curve B also appears to show maximum lipolytic activity with 2% BSA in the assay medium. Further increases in BSA concentration in the assay have little stimulatory or inhibitory effect on the hormone-stimulated lipolytic activity (at least up to 10 per cent).

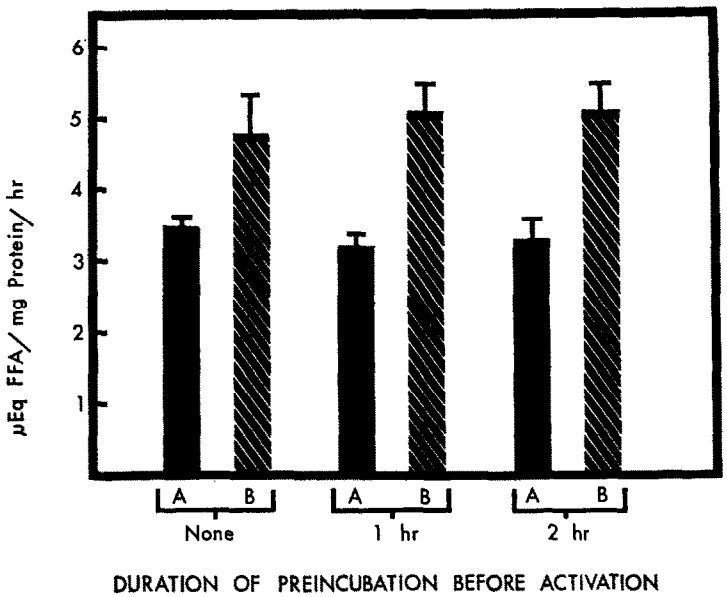


FIG. 4. Effects of varying preincubation period (time between preparation of cells and activation step; see Methods). A represents the lipolytic activity of nonhormone-treated homogenates; B represents the activity of homogenates from cells treated with 10^{-5} NE and 10^{-3} theo prior to disruption. Values given are means of five experiments ± S.E.M. Incubation period was 60 min at 37°.

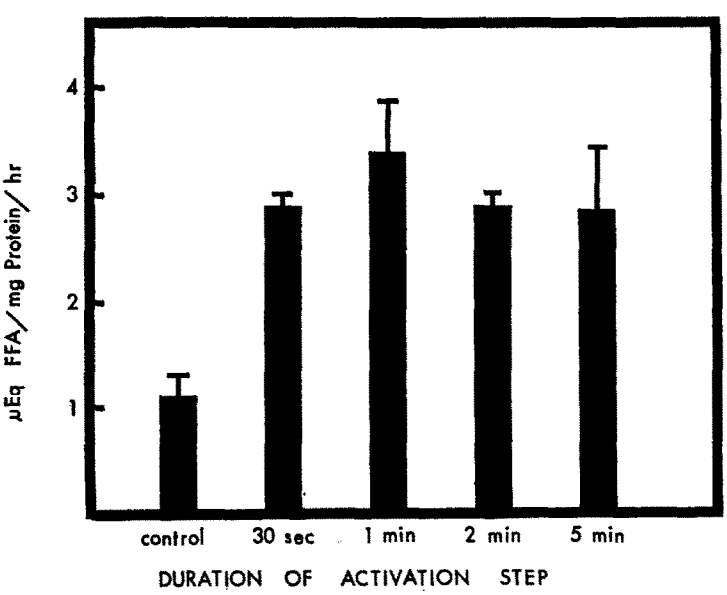


FIG. 5. Effects of varying the duration of activation step (see Methods). Control bar represents nonactivated homogenates. The 30-sec, 1-min, 2-min and 5-min bars represent homogenates from cells treated with 10^{-5} NE and 10^{-3} theo prior to disruption. Values given are the means of five experiments ± S.E.M. Incubation period was 60 min at 37°.

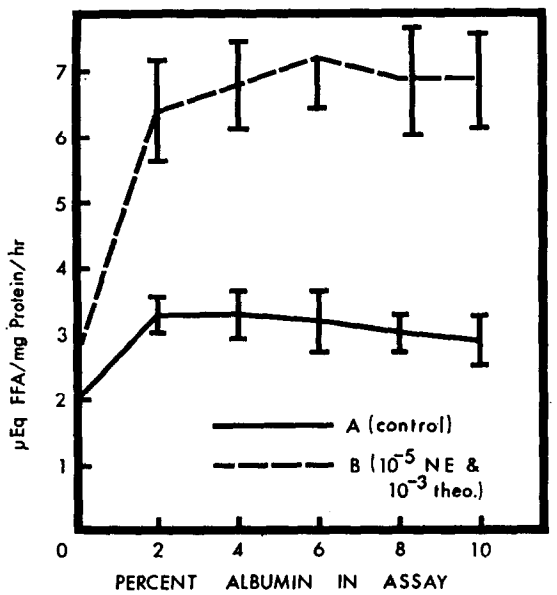


FIG. 6. Effects of varying bovine serum albumin concentration in assay medium on homogenates A and B. NE and theo were administered to homogenate B prior to cell disruption. Points represent the means of five experiments ± S.E.M. Incubation period was 60 min at 37°.

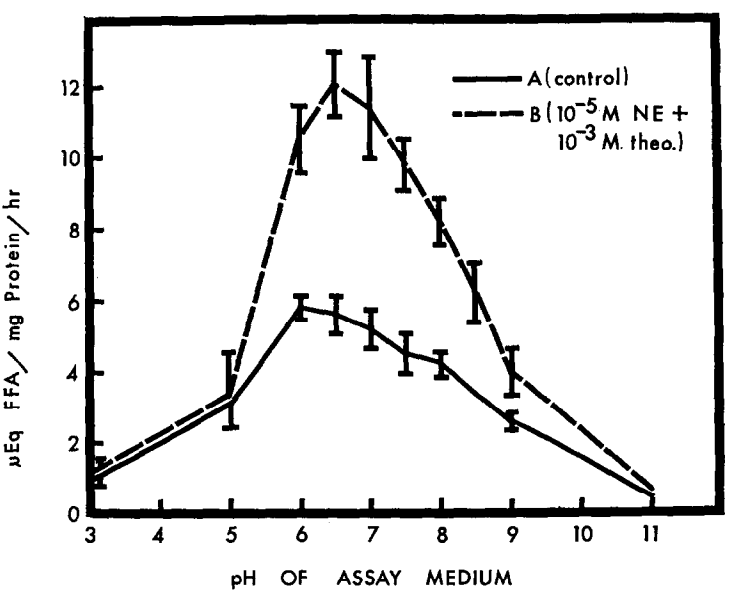
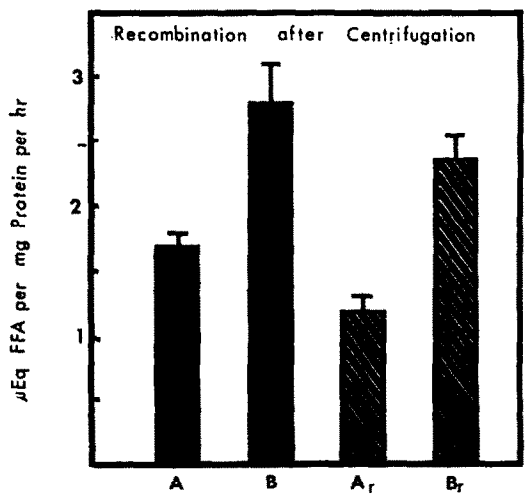


FIG. 7. pH optima of nonactivated (A) and activated (B) broken cell preparations from isolated adipocytes. NE and theo were administered to homogenate B prior to cell disruption (see Methods). Buffers used were citrate, acetate, phosphate, lysine and carbonate. Points represent means of five experiments ± S.E.M. Incubation period was 60 min at 37°.

Figure 7 shows the effects of varying the pH of the assay medium. In these experiments, the final buffer concentration was held constant at 0·02 M while the buffer itself was varied in order to provide maximum buffer capacity. Appropriate blanks were run to rule out any inhibitory effects of the buffer *per se*. Assay pH was varied from 3 to 11. The assay pH was measured at the end of the incubation period and was found to hold constant during the 1-hr period. Both hormone-stimulated and non-stimulated broken cell preparations showed a pH optimum in the range of 6·5–7·5.

In Fig. 8 the results of the recombination, resonication and assay of broken cell preparations after centrifugation at 12,000 *g* for 10 min are presented. Initially, after centrifugation at 0° and recombination, the retention of lipolytic activity was difficult



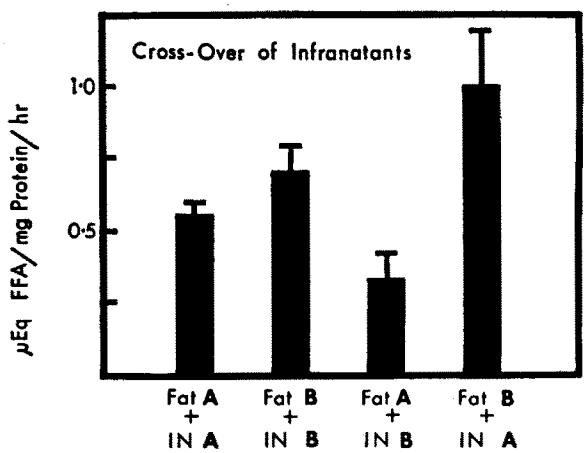
Analysis of Variance	d.f.	F	Sig. Level
A vrs. B	1 & 10	13·25	·01
A_r vrs. B_r	1 & 10	23·72	·01
A vrs. A_r	1 & 10	6·06	·05
B vrs. B_r	1 & 10	2·42	n. s.

FIG. 8. A represents lipolytic activity of control homogenates; B represents activity of homogenates from cells treated with 10^{-5}M NE and 10^{-3}M theo prior to cell disruption; Ar represents activity of homogenates A after centrifugation and recombination; Br represents activity of homogenate B after centrifugation and recombination. Values given are the means of five experiments ± S.E.M. Incubation period was 60 min at 37°.

to obtain; this problem was overcome by centrifuging at 37°. Subsequently, it was found that slight warming of the centrifugate prior to resonication also allowed full recovery of lipolytic activity after recombination. Analysis of variance of these results shows the following: (1) homogenates made from cells treated with NE and theo (B) prior to cell disruption possess significantly greater lipolytic activity over the 1-hr incubation period than do homogenates made from control cells (A); in other words, the B broken cell preparations upon which further experimental procedures were performed were "activated" before such procedures were performed; (2) lipolysis in

activated homogenates after centrifugation and recombination (Br) is significantly different from that of control homogenates after the same procedure (Ar); (3) there is no significant loss of lipolytic activity in the activated (B) homogenates after centrifugation and recombination (B is not significantly different from Br); and (4) there is some loss of basal lipolytic activity after centrifugation and recombination (Ar is significantly lower than A).

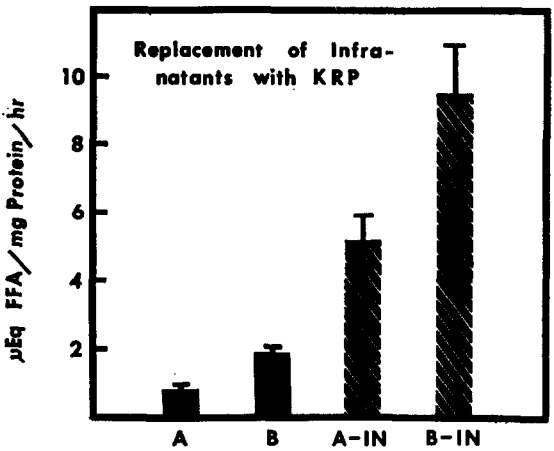
Figure 9 shows the results of the separation and removal of the fat cakes and infranatant fluids of both activated and nonactivated homogenates after centrifugation at 12,000 *g* for 10 min, and the subsequent recombination and assay of the homogenates in the manner discussed above. It can be observed that NE and theo-stimulated



Analysis of Variance	d. f.	F	Sig. Level
IN A vrs. IN B	1 & 14	2·49	n. s.
Fat A vrs. Fat B	1 & 14	4·60	·05

FIG. 9. Results of crossover of infranatants procedure. A represents control homogenates; B represents homogenates from cells treated with 10^{-5}M NE and 10^{-3}M theo prior to cell disruption. IN stands for infranatant fluid. Values given are the means of five experiments ± S.E.M. Incubation period was 60 min at 37°.

lipolytic activity (B) is retained after recombination of fat A and fat B with their own respective infranatant fluids (B > A at 0·01 level). The recombination and assay of fat A plus infranatant B, however, resulted in lipolytic activity which was lower than that of fat A plus infranatant A. In contrast, the recombination and assay of fat B plus infranatant A resulted in lipolytic activity greater than that of fat B plus infranatant B. Analysis of variance shows that the NE and theo-stimulated lipolytic activity is associated with fat cake B, since those combinations with fat B present were significantly greater than those with fat A present (line 2, analysis of variance table, Fig. 9); while those combinations with infranatant B present were not different from those combinations having infranatant A present (line 1, analysis of variance table, Fig. 9).



Analysis of Variance	d.f.	F	Sig. Level
A vrs. B	1 & 8	13·95	·01
A-IN vrs. B-IN	1 & 8	12·31	·01
INpresent vrs. INreplaced	1 & 18	61·14	·01

FIG. 10. Results of the replacement of infranatant fluids with KRP. A represents the lipolytic activity of control homogenates; B represents the activity of homogenates from cells treated with 10^{-5}M NE and 10^{-3}M theo prior to cell disruption; A-IN represents the activity of homogenate A with infranatant fluid replaced by an equal volume of KRP; B-IN represents the activity of homogenate B with infranatant fluids replaced by KRP. Values given are means of five experiments ± S.E.M. Incubation periods were 60 min at 37°.

The results of the replacement of the infranatant fluids with KRP after centrifugation of activated and nonactivated homogenates are presented in Fig. 10. It can be seen that the NE and theo-stimulated lipolytic activity is retained after the replacement of infranatant fluids with KRP. The apparent lipolytic activities of both the A and B broken cell preparations are increased by the removal of their infranatant fluids. Analysis of variance shows: (1) that activated (B) preparations are different from nonactivated (A) prior to removal of the infranatant fluids; (2) that preparation B minus the infranatant fluid is still significantly different from preparation A minus the infranatant fluid; and (3) broken cell preparations (both A and B) with their infranatant fluids replaced show significantly greater lipolytic activity than such preparations with their infranatant fluids present.

DISCUSSION

A variety of methods for both initiating and assaying hormone-stimulated lipolytic activity in rat adipose tissue are present in the literature. Many of the procedures involve modifications and manipulations of the activation or assay steps employed or of both steps; several of these procedures have proved difficult to reproduce. It is essential, therefore, before any further purification and characterization of the 'hormone-sensitive lipase' in rat adipose tissue is undertaken, to develop a clearly defined, easily reproducible means of obtaining and assaying this lipolytic activity.

The results presented above indicate that the liberation of FFA from broken cell preparations made from both control and hormone-treated adipocytes is linear over a 1-hr incubation period. Such an incubation time is consistent with that used on whole fat pads by Vaughan and Steinberg,[14] and by Wenkeová and Mosinger.[15] Likewise, the fact that the liberation of FFA by isolated adipocytes is linear over a 1-hr period has been demonstrated by Lech and Calvert[11] and by Allen *et al.*[16] Similarly, Vaughan *et al.*[4] have reported that the production of FFA by homogenates made from whole fat pads is linear for at least 40 min.

The total lipolytic activity of the broken cell preparations of adipocytes represents approximately 75 per cent of the activity obtained from whole cells treated in a similar manner.[16] The activity reported herein is, thus, much greater than that previously reported in the literature. The 25 per cent difference in lipolytic activity between whole cells and broken cell preparations seen, however, may possibly be due to (1) the stimulation of an inactivating system for the activated lipolytic system; (2) mechanical denaturation of the enzyme(s) during homogenization; or (3) thermal denaturation during sonication or assay.

On the basis of the effective concentrations observed, the NE and theo dose–response curves presented in this paper are somewhat similar to those reported by Allen *et al.*[16] for whole adipocytes. It appears, however, that only the second peak of the biphasic response[16] of adipocytes to NE is present after cell disruption. It is possible, therefore, that this second phase represents the hormonal stimulation of the enzymatic hydrolysis of triglyceride, while the first phase may consist of the stimulation of some other related process, such as fatty acid transport or inhibition of esterification of FFA. Overall, the hormonal stimulation of lipolysis appears to be a more complex process than the simple conversion of an inactive to an active enzyme.

It has been reported by Vaughan *et al.*[4] and Rizack[3] that the basal lipolytic activity of isolated fat pads declines during a preincubation period without hormone. Such does not appear to be the case with broken cell preparations from isolated fat cells. The results shown in Fig. 4 indicate that a preincubation period of up to 2 hr after cell preparation, prior to activation, does not lower the basal, nonstimulated lipolytic activity of this preparation. Immediate activation of the cells after preparation is therefore possible with no reduction of the apparent stimulatory effect of the hormone. A possible explanation for this observation is that the time required for the preparation of isolated adipocytes may actually represent the preincubation period which appears to be necessary for broken cell preparations made from whole tissue.

The pH optimum for lipolysis in this broken cell preparation appears to be similar to the optimum of 7·5 reported for homogenates of whole fat pads.[14] It is different, however, from the pH optimum of 8·5 reported for whole fat pads by Wenkeová and Mosinger.[15] Such a difference in pH optimum between whole fat pads and broken cell preparations is not necessarily contradictory, since the activity of the whole pad may represent additional triglyceride lipolysis by an enzyme or enzymes not present in isolated cells. The response of the above mentioned broken cell preparation from isolated fat cells to albumin in the assay medium is, however, similar to that which is already well documented in the literature.

The localization of hormone-stimulated lipolytic activity after centrifugation of homogenates of adipose tissue has been the object of several recent investigations. Thus, according to the specific methods employed, hormone-stimulated lipolytic

activity has been found associated with the oil layer, the fat cake, the infranatant fluid and the pellet obtained from such centrifugations. Table 1 presents a summary of this work. It includes two methodological factors of importance: (1) the medium in which the adipose tissue was homogenized, and (2) the force and duration of the centrifugation (when available).

It can be seen from Table 1 that no simple relationship between the localization of lipolytic activity and the homogenizing medium used and/or duration and force of centrifugation is evident. Rather, it appears that both factors play an important role

TABLE 1. RELATIONSHIP OF LOCALIZATION OF HORMONE-STIMULATED LIPOLYTIC ACTIVITY TO THE TYPE OF HOMOGENIZING MEDIUM USED AND THE FORCE AND DURATION OF CENTRIFUGATION

Reference	Homogenizing medium	Centrifugation Force (*g*)	Centrifugation Duration	Location of lipolytic activity
5	KRB	18,000	30 min	Infranatant
8	0·1 M PO_4	15,000	30 min	Fat cake
9	0·06 M PO_4	105,000	2 hr	Oil layer (upper)
3	0·25 M sucrose	12,000	10 min	Infranatant
3	0·25 M sucrose	105,000	12 hr	Pellet
6	Saline	105,000	18 hr	Pellet
4	0·154 M KCl	15,000	10–30 min	Fat cake
17	Ion-free medium	105,000	1 hr	Supernatant
18	0·15 M KCl	15,000		Supernatant

in the localization of hormone-stimulated lipolytic activity during centrifugation. It appears that at forces greater than 15,000 *g*, lipolytic activity is found associated with the infranatant or pellet regardless of the homogenizing medium used. At lesser centrifugal forces, however, such as that reported herein and that employed by Rizack,[3] the localization of lipolytic activity appears to depend on the nature of the homogenizing medium: with a medium containing salts (such as KRP) the lipase is found associated with the fat cake as described above; with an ion-free medium, (such as sucrose), however, the lipase appears to localize in the infranatant fluid.

At centrifugal forces greater than 100,000 *g*, the hormone-stimulated lipolytic activity is found in the pellet, except in the system reported by Hollett[9] in which such activity was detected in the uppermost oil layer. This localization of lipolytic activity may again depend on the nature of the homogenizing medium used, since the phosphate buffer used by Hollett was less concentrated than that used by the other investigators. It should be noted, however, that in attempting to compare the published findings from several laboratories, as is done in Table 1, some of the many other differences in methodology (for example, starting with fat pads or fat cells, method and temperature of homogenization, and assay system for "lipase" activity) may be of equal or greater importance than the two factors discussed herein.

In none of the above mentioned systems has both basal and hormone-stimulated lipolytic activity been followed simultaneously. In the work described herein, however, both nonstimulated and hormone-treated broken cell preparations from isolated adipocytes have been studied. After homogenization in KRP buffer and centrifugation at 12,000 *g* for 10 min, both basal (nonstimulated) and catecholamine-stimulated

lipolytic activities were found to be associated with the fat cake. Two possible explanations for these observations are as follows: (1) the hormone-sensitive lipase in both inactive and active forms is saturated with substrate and thus is highly lipophilic, as suggested by Vaughan *et al.*[4] or (2) the hormone-stimulated lipase is a lipoprotein, and as such, has a high affinity for lipids, as reported by Huttenen *et al.*[17]

Furthermore, the infranatant fluid from the hormone-treated preparation appeared to be somewhat inhibitory on basal lipolytic activity (Fig. 8), while the complete removal of the infranatant fluid and replacement with equal volumes of KRP resulted in a great increase in both basal and catecholamine-stimulated lipolytic activity. These two observations are consistent with the possibility that the infranatant fluid contains a substance or enzyme system which is inhibitory to both the basal and stimulated lipolytic activity.

The replacement of the infranatant solutions with KRP also served as an initial step in the purification of the 'hormone-sensitive lipase'. This procedure resulted in approximately a 5-fold increase in the specific activity of both basal and hormone-stimulated preparations. Work is currently being undertaken to purify more completely this enzyme or enzymes.

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