The Characterization of a New Lipolytic Enzyme in Pancreatic Extracts

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Studies by Saxl (1957*a*, 1960) and Hall (1958) on the production of clearing factor in tissues and in plasma, both *in vitro* and *in vivo*, have demonstrated that the enzyme E_1 (Hall, 1957) or a closely related component of the elastase complex may play a part in the formation of a lipoprotein lipase. The properties of this enzyme were therefore studied with a view to characterizing it in relation to other enzymes of pancreatic origin with this type of activity.

Recent studies on the nature of post-heparin lipoprotein lipase have demonstrated that the production of this enzyme is associated with the appearance of variable amounts of other enzymes with different esterolytic properties. For instance, Levy & Swank (1954) showed that the injection of heparin into a dog is followed by the appearance in the plasma not only of clearing factor but also by an increase in tributyrinase, whereas there is no significant increase in this enzyme after a similar injection into a human subject (Levy & Swank, 1955). Similarly, Skorepa & Todorovicova (1958), using olive oil, ethyl butyrate and amyl acetate as substrates, found little significant rise in lipase activity but a 50% rise in *ali*-esterase activity after the intravenous administration of heparin. Since the present study was completed, Pav & Wenkeova (1960) have shown that a comparison of the activity of post-heparin plasma on Tween and on a triglyceride emulsion (Ediol) indicates that in addition to *ali*-esterase, at least two lipases are present in post-heparin plasma, one of which can hydrolyse Tween.

Methods that have been employed for the assessment of lipoprotein-lipase activity have ranged from the direct measurement of the decrease in extinction of lipaemic serum by which it was first recognized (Hahn, 1943; Anderson & Fawcett, 1950) to an estimation of the unesterified fatty acids (Shore, Nicholls & Freeman, 1953) or glycerol (Korn, 1955) that are released during lipolysis. Studies of the kinetics of lipolytic reactions with such methods are, however, hampered by the fact that the enzyme and the substrate are in separate phases. Gomori (1945) first suggested the use of Tween compounds (the polyoxyolefin sorbitan

esters of long-chain fatty acids) as suitable substrates for esterases and lipases and this possibility was elaborated by Archibald (1946) and Copenhagen, Stafford & McShan (1950). Although the solubility of the fatty acids is low, they may remain in solution at the optimum pH (about pH 8) and so affect the course of the reaction. It is therefore of interest that Katz (1957) was able to develop the turbidimetric method, originally devised by Gomori (1949), in which the products of the reaction are removed from solution by the formation of calcium soaps. By the use of an improved version of this technique it has proved possible to characterize the component of the elastase complex which is concerned in clearing-factor production and to differentiate it from pancreatic lipase.

MATERIALS AND METHODS

Enzyme preparations. A partially purified preparation of pancreatic lipase was obtained from defatted hog-pancreas powder (Viobin Corp., Monticello, Ill., U.S.A.) by the method of Sarda, Marchis-Mouren, Constantin & Desnuelle (1957). This material was freeze-dried and in such a state retained its activity indefinitely if stored at 4°. In agreement with the original authors it was found to be unstable in the absence of substrate, especially when incubated in solution at 37°. During the preincubation of enzyme solutions to bring them to the desired temperature before mixing with substrate, periods as short as 10 min. were sufficient to decrease the activity of the system by 50%.

The other pancreas extract employed in these studies (Hall, 1958 and unpublished work; Saxl, 1960) consisted of that fraction of a crude elastase preparation which remained after the dialysis of an acetate buffer, pH 4.7, extract of whole defatted pancreas powder (Hall & Czerkawski, 1959). This acetate extract-soluble material (AES) contains a small amount of a substance which has esterase activity as measured by its liberation of β -naphthyl laurate, but it has been shown (D. A. Hall, unpublished work) that this is not the major lipolytic component and appears to consist of the lipase mentioned above.

Protein preparations. Two different types of protein have been employed. Whole dried human plasma was used for the preliminary experiments and lipoprotein fractions for the later series. The latter were prepared from pooled normal human plasma by ultracentrifuging by the method of Hillyard, Entenman, Feinberg & Chaikoff (1955), and the fractions represented a chylomicron-rich component and high- and low-density-lipoprotein components.

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Substrate. In the present study Tween 20 has been used throughout. On the basis of its laurate content the molecular weight was estimated at about 1500. Owing to the physical nature of the material, substrate preparations were calculated by weight, and the substrate concentration was determined from the laurate content of the resulting solution, obtained after saponification with 0.1N-sodium hydroxide. The amount of laurate liberated showed a maximum after saponification for 15 min. This may be due to a breakdown of the remainder of the molecule after prolonged heating with alkali to produce substances which interfere with the subsequent laurate estimation. Hence the estimated laurate content of the Tween compound (0.6 μ mole/mg.) may be in error. Repetition of the saponification, however, gave consistent results and this method was standardized for characterization of the substrate.

Standardization curves were prepared in the presence of each batch of substrate from 1 to 8 μ moles of sodium laurate. Over most of this range the relationship was linear through the origin and reproducible, and the reaction between laurate and calcium appeared to be complete within 7 min. (The lag periods reported below cannot therefore be due to delays in precipitate formation.)

In most cases it was found expedient to add the calcium chloride to the substrate before the reaction commenced. Because the ensuing precipitate of calcium laurate might adsorb components of the system or the products of the reaction, it was necessary at the outset to determine whether this procedure gave results which differed from those obtained when the reaction was permitted to proceed in the absence of calcium, which was then added at the completion of the reaction. The results of 20 estimations

carried out by both methods showed no statistically significant difference ($P < 0.01$).

Measurement of opacity. To obtain optimum readings of extinction Katz (1957) advocated that the reaction mixture should be cooled. This was found to be inadvisable since, although the extinction at 37° was 30% less than at 18°, it was difficult to determine accurately the exact period of incubation if extinctions were read throughout the course of a reaction which was interrupted by repeated cooling and reheating cycles.

The extinction was measured on a Unicam model SP. 400 diffraction-grating spectrophotometer. The wavelength at which the extinction was read was raised from 550 $m\mu$ (Katz, 1957) to 662 $m\mu$ to permit a study of the inhibition of the reaction by eserine, since all commercial preparations of eserine sulphate examined developed a marked pink when incubated either alone or in the presence of the reaction mixture at concentrations above 10 mM for periods of more than 1 hr.

Choice of buffer. Fig. 1 shows two series of curves representing the time course of the reaction between the pancreatic extract AES and the substrate at increasing periods of time after the initial preparation of the substrate. The two series represent the results obtained in 0.2M-tris (2-amino-2-hydroxymethylpropane-1:3-diol) (Katz, 1957) and 0.2M-ammediol (2-amino-2-methylpropane-1:3-diol) buffers. The change in extinction of the untreated substrate is less with the ammediol solution than with tris within a few days of its preparation. Thereafter, however, the ammediol substrate tends to deviate from its initial state more rapidly. During the first 10 or 15 min. of incubation in the presence of tris buffer a decrease in

Fig. 1. Time course of the early stages of the reaction between 6 mg. of the pancreatic extract AES and the substrate (Tween) (12 mM) at various times after the preparation of the substrate for systems made up in (a) ammediol and (b) tris buffers. (a) Curves (A)–(F) and (b) curves (A)–(C) are for substrate preparations aged 0, 2, 4, 8, 13 and 16 days and 0, 4 and 16 days respectively.

Table 1. *Effect of age on the response of Tween 20 dissolved in ammediol and tris buffers to the action of the enzyme preparation AES*

Age of prep. (days)	Ammediol		Tris	
	Initial velocity (μmole/min.)	Lag (min.)	Initial velocity (μmole/min.)	Lag (min.)
0	0.067	13	0.080	19
1	0.070	12.5	—	—
2	0.085	12.0	0.077	13
3	0.095	10.2	0.075	11
4	0.104	7.5	0.070	10
5	0.107	5.0	—	—
7	0.105	3.5	0.055	11
8	0.105	3.0	0.047	11
13	0.100	2.5	0.035	12
15	0.095	2.0	—	—
16	0.091	2.0	0.025	11

Fig. 2. *Effect of substrate concentration [S] on the initial velocity of systems in which the enzymes pancreatic lipase (A) and AES (B) act on Tween 20. Abscissa values are expressed in terms of the logarithm of the initial substrate concentration and the ordinates in terms of μmoles of lauric acid liberated/min.*

Table 2. *Comparison between the lag periods induced in the pancreatic lipase and AES systems by alterations in the concentration of the substrate*

The substrate in this and subsequent experiments was aged between 4 and 10 days before use.

Concn. of substrate (μmoles/l.)	Lag period (min.)	
	Pancreatic lipase	AES
0.1	1.0	13.0
0.2	1.1	8.0
0.4	—	5.0
0.8	1.0	4.0
1.0	0.8	—
1.2	—	4.0
2.0	0.9	3.5
4.0	0.8	3.5
8.0	1.4	4.5
12.0	2.0	7.0
16.0	2.3	8.5
20.0	2.6	10.0

extinction occurs. This results in a lag period before the main course of the reaction supervenes. Although there is a similar lag with substrates dissolved in both tris and ammediol buffers in the first few days after the preparation of the substrate, its length is progressively decreased as the substrate ages. Table 1 shows the greater response of the ammediol substrate to this age-induced decrease in lag. In the second and fourth columns the initial reaction velocity of the main reaction is recorded for substrate preparations of different ages. Since there is a slight induction period before the main reaction commences the 'initial' velocity is calculated from the slope of the steepest tangent to the time curve. The induction period is only one-fifth to one-tenth of the lag and one-tenth of the length of the steady-state reaction; thus such a value is a close approximation to the true initial velocity. To compensate for the use of this value instead of the true initial velocity, other parameters taken into account in the kinetic analysis of these systems are calculated at this same point of maximum velocity, e.g. the substrate concentration at maximum velocity replaces the original substrate concentration. The intercept on the abscissa of the tangent at maximum slope also represents a reasonably accurate assessment of the lag period. These values show that the most suitable conditions for the estimation of lipolytic activity occur when a substrate preparation, dissolved in ammediol, has aged for a period of between 4 and 10 days. The majority of the values recorded below were obtained on substrates prepared in this manner.

Effect of substrate concentration. The curves in Fig. 2 record the initial velocities of systems containing a 240-fold range of substrate concentrations, and Table 2 shows the corresponding lag periods, before the main reaction commences, obtained as described for both AES and pancreatic lipase. For the AES fraction there is no further increase in velocity above a concentration of 6 mM, and at this concentration, also, the lag period is least. A similar peak in the lipase curve occurs at a slightly higher substrate concentration, but there is little lag over the major portion of the range. For comparative purposes it is convenient therefore to employ substrate concentrations in the region of 6 mM because, in view of the shortened lag period, it is possible under these conditions to maintain the incubation time within reasonable limits.

Effect of changing enzyme concentration. Both the enzyme preparations studied were only partially purified, and to obtain changes in extinction that were large compared with the errors of the method, it was necessary to use 6 mg. of freeze-dried enzyme powder to produce an effect comparable with that of 1 ml. of post-heparin plasma. Variations in enzyme concentration produced the effects recorded in Fig. 3. In addition to studies at the optimum substrate concentration, experiments were also carried out at a raised level of 43 mM to determine whether higher enzyme concentrations could reverse the retarding effect of such high substrate concentrations. As can be seen, this does in fact occur, and, at lower enzyme concentrations, in addition to a decreased initial velocity there is an even greater lag period.

Determination of lipase. The method finally devised for the estimation of lipolytic activity is as follows: to 1.5 ml. of 50 mM-ammediol buffer, pH 7.8, was added 0.2 ml. of a 5% (w/v) solution of Tween 20 in 5 mM-ammediol buffer containing 0.2% (w/v) of calcium chloride. After the

addition of a suitable amount of enzyme (also dissolved in ammiediol buffer) and any other reagents which are required, the total volume was made up to 3.5 ml. with concentrated buffer. Incubation was at 37° in loosely stoppered 25 ml. conical flasks. Readings of opacity were taken at 10 min. intervals over the first 2 hr., at $\frac{1}{2}$ hr. intervals for the next 3 hr. and a final reading was taken after 20 hr. This enables very dilute enzyme solutions to be compared under conditions in which, although they are apparently inactive after short periods of incubation, appreciable amounts of fatty acid are liberated in a 20 hr. period.

RESULTS

A number of workers have reported that liver, intestinal and serum lipase differ from post-heparin lipoprotein lipase of plasma, basing their observations on the differing response of the various enzymes to inhibitors and activators. Dissimilarities can also be observed between pancreatic lipase and the fraction AES in this respect. Many of the previously reported findings on the action of various inhibitors on lipoprotein lipase appear, from lack of evidence to the contrary, to have been carried out at single substrate concentrations, thus making it difficult to assess in detail the mechanism whereby inhibition occurs. A fuller study of the reaction between AES and a number of inhibitors has therefore been made and, because this is the first time that such a study has been made of the reaction between pancreatic lipase and a soluble substrate, a similar detailed appraisal of the latter enzyme has been made for comparison.

Fig. 3. Relationship of lag period (●) and initial velocity (○) to the concentration of the enzyme AES (mg./ml.), for two different substrate concentrations: (A) 6 mM; (B) 43 mM.

Table 3. Lag induced in an AES system by various concentrations of eserine at two substrate concentrations

Concn. of eserine (mM)	Concn. of substrate	
	mm	10 mm
	Lag period (min.)	
1	22	3
2	26	4
4	29	4
6	33	6
8	37	8
10	44	11

Fig. 4. Effect of variations in the concentration of enzyme on the inhibition of the system AES-Tween by 10 mM-eserine. (A) Response of the system to increased concentrations of AES; (B) comparable curve in the presence of 10 mM-eserine.

Fig. 5. Plot of $1/v$ for the system pancreatic lipase-Tween (expressed in terms of change in $E/\text{min.}$) against $[I]$ (eserine concentration) from 1 to 10 mM for two concentrations of substrate: (A) 2 mM; (B) 5 mM. From the intersection a value of K_i of 1.7 l./m-mole may be calculated.

Eserine. At concentrations between 1.0 and 10 mM, eserine has little effect on the initial reaction velocity of the AES-Tween reaction. There is, however, a progressive increase in the lag before the main reaction commences. This lag is greater at lower substrate concentrations (Table 3). In view of the relatively large size of the substrate molecules it is possible that the reaction between AES, Tween and eserine might represent competition between the inhibitor and the enzyme for sites on the substrate. Fig. 4 shows that there is a reversal of the inhibition produced by the addition of eserine at enzyme concentrations greater than 1700 mg./l. In contrast with AES, over the same range of concentrations, eserine inhibits the action of pancreatic lipase in a competitive fashion (Fig. 5). A plot of the reciprocal of the initial velocity against inhibitor concentration at two different substrate concentrations in the region of optimum response to substrate shows the typical linear relationship of competitive inhibition with a point of intersection on the left of the ordinate and a value for K_i of 1.7 l./m-mole.

Cetavlon. For this inhibitor also there is a marked difference between the response of the two enzyme preparations. With AES there is an increase only in the lag period, whereas a true decrease of the rate of reaction occurs with pancreatic lipase. The kinetic analysis of this system is, however, not as simple as for one undergoing inhibition by eserine. At low inhibitor concentrations the inhibition appears to be competitive, but there is considerable deviation from linearity in plots of v/v_0 against $[I]$ above an inhibitor concentration of 0.3 mM. This is especially true at a substrate concentration of 6 mM, at which the enzyme shows optimum activity (Fig. 6).

Heparin. The reaction between either of the two enzymes and heparin appears to be even more complex. Its activity on pancreatic lipase is similar in some respects to the effect of Cetavlon in that there is little response at substrate concentrations well away from that at which optimum activity occurs in the absence of inhibitor (Fig. 7). At intermediate substrate concentrations, how-

Fig. 6. Effect of Cetavlon on the activity of pancreatic lipase. Rate of the uninhibited reaction (v_0) is plotted as a fraction of that of the inhibited reaction (v) at each concentration. Curves refer to various substrate concentrations (mM): (A) 1; (B) 2; (C) 3.5; (D) 5.

Fig. 7. Effect of heparin on the activity of pancreatic lipase, expressed as in Fig. 6. Concn. of Tween (mM): (A) 1; (B) 2; (C) 5.

Table 4. Effect of heparin on the time course of the AES system, showing the slight inhibitory effect at high heparin concentrations

Period of incubation (min.)	Concn. of heparin ($\mu\text{g./l.}$)					
	0	170	340	510	680	850
	Lauric acid liberated (m-moles/l. of reaction mixture)					
20	0.28	0.02	0.04	0.20	0.10	0.10
30	0.30	0.04	0.19	0.48	0.14	0.10
50	0.74	0.62	0.65	0.78	0.20	0.18
60	1.40	1.14	1.14	1.56	1.17	1.15
75	1.98	1.62	1.68	2.04	1.54	1.54
90	2.39	2.22	2.26	2.80	2.24	2.10
105	2.50	2.48	2.62	2.90	2.40	2.20
120	2.75	2.80	2.94	3.30	2.80	2.40

ever, at inhibitor concentrations above $170 \mu\text{g./l.}$, there is a marked inhibition.

On the other hand, heparin may act as an activator of AES. Table 4 shows the effect of the addition of increasing concentrations of heparin on the time course of the reaction between AES and

Tween. After an initial drop there is a progressive increase in activity up to a heparin concentration of $510 \mu\text{g./l.}$, although above this level the activity decreases again.

Ionic strength. All the foregoing reactions were carried out in buffer of ionic strength (I) 0.05. Decrease of I to 0.01 induced a 25% increase in activity, whereas its increase to 0.2 caused a fall in activity of a typical AES preparation to 33% of its original value. Higher concentrations were even more effective.

Serum proteins. The addition of serum proteins to systems containing AES results in the introduction of an enhanced lag period (Fig. 8). This lag is, however, followed by rapid lipolysis at a rate commensurate with, or greater than, the original initial rate of the system without added protein. The point at which rapid lipolysis ceases is not attained as quickly, with the result that the two

Fig. 8. Effect of increasing concentration of plasma protein on the activity of the system AES-Tween. Curves (A), (B) and (C) refer to systems to which 0, 1 and 2 mg. of whole dried human plasma/ml. respectively were added.

Fig. 9. Preincubation of Tween with plasma protein and its effect on the activity of systems consisting of AES and Tween. Curves (B), (C), (D) and (E) represent systems in which the substrate was incubated for 0, 2, 4 and 5 hr. respectively, at 37° , with 1 mg. of whole human plasma protein/ml. For comparison, curve (A) is for a system without added plasma protein.

Fig. 10. Enhanced lag period (curve A) followed by increased activity of 6 mg. of AES in the presence of 0.14 mg. of a lipoprotein fraction/ml.; this fraction was obtained by ultracentrifuging (at 30 000g) human plasma, which was layered over a medium of density 1.063 and contained mainly those lipoprotein molecules with flotation constants of S_0 0-20. Curve (B) is a control run without added protein.

curves cross one another and, after 180 min., notwithstanding the longer period of lag, more acid is liberated in the system containing serum protein than in the one containing enzyme alone.

If the substrate is preincubated at 37° with the serum protein, the lag is progressively decreased, although at any one protein concentration there is no increase in the rate of reaction. No decrease is observed after incubation in the absence of protein. Here, as elsewhere in these studies, the rate is taken as the maximum immediately after the cessation of the lag (Fig. 9). The component of the serum which is responsible for this diminution of the lag has not yet been identified, but preliminary observations on the activating function of β -lipoprotein (S_1 0-20) appear to point to the implication of a component of this fraction of the total protein (Fig. 10). Here again there is a lag followed by a rapid period of lipolysis, causing the two curves to cross after a certain proportion of the reaction has been completed. Chylomicra and low-density β -lipoproteins were without similar activity.

DISCUSSION

The concept that a member of the elastase group of enzymes has lipolytic activity is by no means new. Lansing, Rosenthal, Alex & Dempsey (1952) first reported the liberation of droplets having an affinity for Sudan dyes during elastolysis, and on the basis of this suggested that elastase was a lipase. The lipoprotein nature of elastin was reaffirmed by Sacks (1954) and by Labella (1957), the latter again stressing the lipolytic nature of elastase. Saxl (1957a) showed that fractions of elastase preparations which were rich in the component E_1 had the power, in the presence of acid polysaccharide and serum protein, of causing the hydrolytic disruption of lipid in elastic tissue, and Hall (1958) demonstrated that such mixtures formed systems which were similar in properties to the post-heparin clearing factor of plasma. Studies *in vivo* have also been made but with very widely differing results. Lansing (1954, 1959) and Butturini, Langer & Giro (1959) claim that elastase is capable of preventing the formation of atheroma in animals which have been given a cholesterol-rich diet, whereas Tennent, Zanetti, Ott, Kwon & Siegel (1956) report completely negative results for pure elastase. Saxl (1960) and D. A. Hall (unpublished work), on the other hand, have observed that AES, which is virtually devoid of elastase while containing appreciable amounts of E_1 , is capable of bringing about such a preventative action. Since little is known about the purity of the elastase preparations used by Lansing and by Butturini *et al.*, the difference between the results obtained by these various groups of workers may

be due to different concentrations of E_1 in the enzyme preparations which they employed. Saxl and Hall's studies *in vivo* indicated that it was desirable to determine by some independent means whether this could be differentiated from the lipase normally associated with pancreatic extracts.

Tween acts as a substrate for both systems. With the lipase, the effect of increasing the substrate concentration above the value of 6 mM at which optimum initial velocity occurs does not affect the onset of the reaction. With AES, however, there is a considerable increase in the lag period which precedes the reaction. Thus the conditions for the use of Tween as a substrate which are described above would appear to be more suitable than those suggested by Katz (1957) and even more so than those entailing the use of very high Tween concentrations such as were suggested by earlier workers (Archibald, 1946; Copenhagen, Stafford & McShan, 1950). Katz recorded a lag period of similar magnitude for post-heparin lipoprotein lipase without comment.

The lag is prolonged in those systems with relatively lower concentrations of AES, and it may be assumed that this delay in the commencement of the reaction is caused by the setting up of an equilibrium between the enzyme and the active and inactive sites on the substrate molecule. Complexes formed at the latter sites would decrease the effective concentration of the enzyme and result in the prevention of reaction until a sufficient number of enzyme molecules become adsorbed on to active centres.

The shapes of the curves in Fig. 2 indicate that both enzyme systems are inhibited by the substrate and this is borne out by the Lineweaver-Burk plots for these data as presented in Fig. 11. In these, good linearity is apparent in those portions of the curves which correspond to low concentrations of substrate. The concentration at which deviation from linearity occurs differs in the upper two curves, which refer to AES at two different enzyme concentrations, from that shown in the lower one, which is for pancreatic lipase. Laidler & Hoare (1949) have shown that such inhibition by substrate can be expressed by equation 1.

$$\frac{1}{v} = \frac{1}{K_2 K_s [E]_0 [S]} + \frac{K_s + K_i}{K_2 K_s [E]_0} + \frac{K_s K'_i}{K_2 K_s [E]_0} [S], \quad (1)$$

v , $[E]_0$ and $[S]$ refer to velocity, enzyme concentration and substrate concentrations respectively and the constants are: K_s , equilibrium between enzyme and active enzyme-substrate complex; K_i , equilibrium between enzyme and inactive enzyme-substrate complex; K'_i , equilibrium between active enzyme-substrate complex and inactive enzyme-substrate complex containing two substrate mole-

cules, cf. Laidler & Hoare (1949); K_s , the overall reaction. From equation 1, the substrate concentration at which the reciprocal of the initial velocity is a minimum can be determined by differentiation.

$$\frac{d(1/v)}{d(1/[S])} = \frac{1}{K_s K_s [E]_0} - \frac{K_s K'_i}{2 K_s K_s [E]_0} [S]^2. \quad (2)$$

By equating $[d(1/v)/d(1/[S])]$ to zero it can be shown that the value of $[S]$ at the minimum is independent of enzyme concentration, being expressed by equation 3.

$$[S] = \sqrt{\frac{2}{K_s K'_i}}. \quad (3)$$

This deduction can be verified from the curves in Fig. 11. In the upper ones, which demonstrate the effect of different concentrations of AES, there is no difference in the position of the minimum with respect to values of $1/[S]$, although the slopes of the linear portions, which are dependent on elastase concentration, vary. The fact that the minima for the AES curves differ from that for the pancreatic lipase indicates that the two enzymes are different, since the value of $[S]$ at this point is dependent solely on constants which are characteristic of the enzyme concerned (cf. equation 3).

The differences between the response of the two enzymes to the various inhibitors present even more concrete evidence in favour of the separate existence of two enzymes in pancreas, each capable of bringing about the hydrolysis of the ester linkages of Tween. In general the observations on pancreatic lipase confirm those obtained by other workers with other substrates. However, this is not true of the effect of heparin at higher concen-

trations, especially at those enzyme-substrate ratios which are associated with optimum activity.

The factor of greatest interest, however, is the effect of the various inhibitors on the lag which is apparent in the system AES-Tween.

It appears most likely that those inhibitors which prolong the lag period with the enzyme AES may do so by attaching themselves to the enzyme and thus decreasing the effective concentration of the enzyme rather than by competing with the substrate for sites on the enzyme. On the other hand, proteins, which, after incubation with the substrate, diminish the lag period may do so by attaching themselves to these inactive sites on the substrate, which by adsorbing the enzyme temporarily decreases its effective concentration. Higher concentrations of protein, however, also affect the initial velocity of the reaction and thus demonstrate a similar effect to that of lipoprotein added to systems containing post-heparin or tissue-lipoprotein lipase (Korn, 1958) and triglyceride emulsions.

The differential effect of various heparin concentrations is also indicative of the similarity between the active component of AES and lipoprotein lipase. Korn (1957) has suggested that heparin may constitute an essential component of the lipoprotein-lipase system and inhibition by high concentrations of heparin may be due to competition for specific sites on the lipoprotein molecule between free heparin and that which is bound to the enzyme. Under conditions in which the concentrations of heparin and AES are such that all the sites on the enzyme molecule are not occupied, increasing concentrations of heparin will result in the formation of linkages between the enzyme and the lipoprotein and hence will result in greater activity. At higher heparin concentrations, however, above the threshold value at which all the sites in the enzyme are occupied by heparin, further increases will result in inhibition, owing to the presence of repulsive forces between heparin molecules bound to enzyme and substrate.

The non-specificity of Tween as substrate for lipoprotein lipase prevents any deductions about the possible identity of the active factor in AES with post-heparin lipoprotein lipase on the basis of these observations alone. When taken in conjunction with the results of an examination of the clearing-factor activity of AES (Hall, 1958 and unpublished work) more definite hypotheses can be formed. It is demonstrated that the E_1 component of AES together with a limited concentration of acid polysaccharide and plasma β -globulin can act in exactly the same fashion as post-heparin lipoprotein lipase on lipaemic plasma and on artificial triglyceride substrates with the liberation of unesterified fatty acids.

Fig. 11. A plot of $1/v$ (sec./ μ mole) against $1/[S]$ (ml./m-mole): (A) AES (2 mg./ml.); (B) AES (0.5 mg./ml.); (C) lipase (0.5 mg./ml.).

The possible lipoprotein-lipase activity of AES and the inhibitory effect of heparin may afford one explanation of the apparently paradoxical situation that a preparation (AES) which has been shown (Hall & Czerkawski, 1959) to contain the enzyme E_1 as its major active component appears to have lipase activity when tested against fatty acid esters in the presence of protein and polysaccharide, whereas in the presence of the proteolytic enzyme elastase it releases polysaccharide from elastic tissue and therefore appears to have mucase activity (Hall, 1957; Saxl, 1957b).

Saxl (1960) has shown by the electron optical examination of elastic tissue that it contains lipoprotein. Yu & Blumenthal (1958) have also shown that acid polysaccharide can be made to attach itself to elastic tissue, and it is not unlikely in view of the suggestions made above that it may be through the lipoprotein component of elastin that such an attachment is made. The degradation of such lipid would then result in the release of polysaccharide into solution.

SUMMARY

1. By an adaptation of the method of Katz (1957) for the measurement of esterolytic activity with Tween as substrate, the active component of soluble material from an acetate extract of pancreas (AES) and normal pancreatic lipase are compared.

2. Whereas eserine between concentrations of 1 and 10 mM acts as a competitive inhibitor of pancreatic lipase, it is without effect on the rate of reaction of preparation AES with Tween. It does, however, increase the lag period before the reaction commences.

3. Comparable differences between the two enzymes can be observed after inhibition studies with Cetavlon and heparin.

4. Similarities can be shown to exist between preparation AES and lipoprotein lipase in their reaction with heparin, high concentrations of salt and certain serum-protein fractions.

5. The suggestion is made that the release of polysaccharide, which is associated with the interaction between preparation AES and elastic tissue in the presence of elastase, may be due to the degradation of a lipid moiety of elastin with which the polysaccharide is associated.

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