XXV. THE INFLUENCE OF VARIOUS SUBSTANCES ON LIPASE ACTION¹.

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ROSENHEIM [1910] has investigated the influence of various compounds on the hydrolysis of olive oil by pancreatic lipase. Although no numerical data are submitted, he states that lecithin is inert towards lipase and also that cholesterol retards lipase action. In a previous paper [Corran and Lewis, 1928] results of experiments were recorded which demonstrated that lead, in both the ionic and colloidal form, is capable of increasing the lipolytic augmentation produced by serum, both normal and cancerous. In the present paper, results are submitted on the influence of the chlorides of sodium, potassium and calcium, and of purified lecithin, cholesterol, lead and copper salts on lipolysis in the absence of serum, and the effects of cholesterol, copper and mercury on lipolysis in presence of serum.

The method employed was identical with that outlined in an earlier paper, namely, mixing the glycerol extract of lipase, serum, water (or salt solution) and olive oil emulsion, the latter consisting of 4 parts of purified olive oil to 1 part of 1 % aqueous sodium oleate, and allowing the reaction to proceed at 37° until equilibrium was attained.

I. THE INFLUENCE OF LECITHIN AND CHOLESTEROL ON LIPOLYSIS.

As mentioned previously Rosenheim found that lecithin is inert towards lipase, but that its products of hydrolysis are strong augmentors. In the present work a purified lecithin has been employed, in order to ensure that any effect observed is due to lecithin, and not to impurities². A fine suspension in water was used, of strength 0.01 *M*. Table I gives the mean results obtained.

It is evident that the augmentative effect due to lecithin is small compared with those produced by serum and sodium oleate (for example, 1 cc. of serum and 1 % sodium oleate give augmentations of 20 cc. and 13 cc. respectively [Corran and Lewis, 1928]), the greatest effect being observed with 1 cc. 0.01 *M* lecithin suspension. Incidentally, the results recorded in Table I confirm the conclusions reached by Rosenheim, that the effect of lecithin on lipase is negligible.

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² The writer's thanks are due to Mr H. I. Price, of the Dept. of Physical Chemistry, University of Liverpool, for the sample of pure lecithin, prepared by Levene's method [1927].

Table I.

Duration of experiment, 48 hrs. Temperature 37°.					
Lipase extract cc.	0.01 <i>M</i> lecithin cc.	Water cc.	Olive oil emulsion cc.	<i>N</i> /10 NaOH required cc.	Augmentation in <i>N</i> /10 NaOH cc.
1	0.0	5.0	5	27.90	—
1	0.5	4.5	5	28.20	0.30
1	1.0	4.0	5	34.10	6.20
1	2.0	3.0	5	33.80	5.90
1	3.0	2.0	5	31.20	3.30
1	5.0	0.0	5	30.00	2.10

Considering now the influence of cholesterol on lipolysis, Shaw-Mackenzie [1910] concluded that cholesterol opposes the augmentation produced by sodium cholalate and it may be inferred that cholesterol acts as an inhibitor towards lipase. The influence of cholesterol was therefore investigated.

A purified cholesterol¹ was used, in the form of a coarse suspension in water, strength 0.01 *M*, from which the cholesterol separated on standing, and which was vigorously shaken before the requisite volumes were measured out. A 0.01 *M* solution in ether was also employed. In the latter case, after measuring out the requisite quantities of ethereal solution, these were added to the lipase extract, the ether was allowed to evaporate and water and emulsion were finally added. Table II *a* gives the results using a coarse aqueous suspension and Table II *b* the results employing an ethereal solution of cholesterol, in the absence of serum.

Table II *a*.

Duration of experiment, 48 hrs. Temperature 37°.					
Lipase extract cc.	0.01 <i>M</i> cholesterol cc.	Water cc.	Emulsion cc.	<i>N</i> /10 NaOH required cc.	Effect due to cholesterol in <i>N</i> /10 NaOH cc.
1	0.0	5.0	5	24.25	—
1	0.5	4.5	5	25.30	+ 1.05
1	1.0	4.0	5	26.95	+ 2.70
1	2.0	3.0	5	26.90	+ 2.65
1	3.0	2.0	5	26.35	+ 2.10
1	5.0	0.0	5	25.25	+ 1.00

Table II *b*.

1	0.0	5.0	5	24.75	—
1	0.5	4.5	5	25.30	+ 0.55
1	1.0	4.0	5	26.10	+ 1.35
1	2.0	3.0	5	29.90	+ 5.15
1	3.0	2.0	5	29.55	+ 4.80
1	5.0	0.0	5	25.90	+ 1.15

It is clear that, so far from acting as an inhibitor, cholesterol acts as an augmentor in the concentrations employed, although, as in the case of lecithin, the effects are small compared with those produced by serum. The maximum effect in Table II *b* (5.15 cc. augmentation for 2 cc. cholesterol solution) is

¹ The writer desires to express his thanks to Dr W. A. Sexton, Dept. of Organic Chemistry, University of Liverpool, for the sample of purified cholesterol.

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considerably greater than that obtained from Table II *a* (2.70 cc. augmentation for 1 cc. cholesterol suspension). The variation must be attributed to the unsatisfactory nature of the coarse suspension employed in Table II *a*.

In view of Shaw-Mackenzie's results on the influence of cholesterol on lipolysis in presence of sodium cholalate, experiments were carried out in presence of normal ox serum. The mean results of these experiments are given in Table III. The duration of experiment was 48 hours, corresponding to the maximum extent of hydrolysis observable.

Table III.

Lipase extract cc.	Ox serum cc.	0.01 <i>M</i> cholesterol cc.	Water cc.	Emulsion cc.	<i>N</i> /10 NaOH required cc.	Effect due to cholesterol in <i>N</i> /10 NaOH cc.
1	0.0	0.0	5.0	5	26.50	—
1	0.5	0.0	4.5	5	48.50	—
1	0.5	0.5	4.0	5	49.50	+ 1.00
1	0.5	1.0	3.5	5	48.00	— 0.50
1	0.5	2.0	2.5	5	47.50	— 1.00
1	0.5	4.5	0.0	5	46.50	— 2.00

The effects due to cholesterol are again small. In low concentration of cholesterol an augmentative effect is observed, changing, in higher concentrations, to an inhibitory effect. These results differ from those of Shaw-Mackenzie in that he found a strong inhibitory action by cholesterol in all concentrations. It may be pointed out that the duration of Shaw-Mackenzie's experiments from 2–18 hours gives a measure of the rate of reaction, as the writer found that 48 hours was the minimum period of reaction in order that equilibrium be established. The results of Shaw-Mackenzie and those recorded in the present paper are therefore not strictly comparable. It is evident, moreover, that cholesterol and lecithin play little part in the lipolytic co-enzymic activity of serum.

II. THE INFLUENCE OF SODIUM, POTASSIUM AND CALCIUM CHLORIDES ON LIPOLYSIS.

A great deal of doubt exists as to the influence of the salts of the alkali metals and the alkaline earth metals on lipase action. Tanaka [1912] states that neutral salts of alkali metals augment lipolysis and that salts of the alkaline earths (Ca, Ba, Mg) act as inhibitors. Mellanby and Woolley [1914] state that electrolytes have no effect on the action of pancreatic steapsin. Falk [1918] concludes that sodium salts accelerate¹ but do not augment,

¹ An accelerator is here defined as a substance capable of increasing the *rate* of reaction, no account being taken of its influence on the extent of reaction. An augmentor is a substance capable of increasing the *extent* of reaction, no account being taken in this case of its influence on the rate of reaction: see footnote in previous paper by Corrnan and Lewis [1928]. The extent of hydrolysis observed at periods longer than 48 hours is the same as that observed at 48 hours, corresponding to a limiting extent of hydrolysis. A mass action equilibrium is, however, not assumed.

whilst calcium salts retard the rate and also diminish the extent of lipolysis. Hamsik [1911, 1915], Pekelharing [1912] and Willstätter [1923] state that sodium and calcium salts accelerate lipase action. With a view to determining the influence of the salts of the metals mentioned on the extent of lipase action, varying quantities of the salts were added to the reaction mixture and their effects investigated. The concentration of salts employed was in all cases low, 0.01 *M* aqueous solutions being used. The addition of salts was found to reduce the stability of the olive oil emulsion employed and it was found necessary to shake the reaction vessels at frequent intervals to ensure complete emulsification of the olive oil. The results are recorded in Table IV.

Table IV.

Duration of experiment, 48 hrs. Temperature 37°.							
Lipase extract cc.	0.01 <i>M</i> NaCl cc.	0.01 <i>M</i> KCl cc.	0.01 <i>M</i> CaCl ₂ cc.	H ₂ O cc.	Emulsion cc.	N/10 NaOH required cc.	Effect due to added salts in N/10 NaOH cc.
1	0.0	0.0	0.0	5.0	5	24.00	—
1	0.5	0.0	0.0	4.5	5	24.30	+ 0.30
1	1.0	0.0	0.0	4.0	5	25.90	+ 1.90
1	2.0	0.0	0.0	3.0	5	28.20	+ 4.20
1	0.0	0.5	0.0	4.5	5	24.80	+ 0.80
1	0.0	1.0	0.0	4.0	5	25.30	+ 1.30
1	0.0	2.0	0.0	3.0	5	27.80	+ 3.80
1	0.0	0.0	0.5	4.5	5	21.70	- 2.30
1	0.0	0.0	1.0	4.0	5	19.50	- 4.50
1	0.0	0.0	2.0	3.0	5	14.60	- 9.40

It is evident that sodium and potassium chloride solutions oppose the action of calcium chloride. As these salts constitute the greater part of the inorganic salt constituent of serum, and moreover occur in small quantity, it is clear that the dialysable constituents of serum will have little measurable augmentative effect on lipase. The results with calcium chloride are interesting in view of Willstätter's observation that calcium chloride accelerates lipase action. The difference in reaction conditions, for example p_H of the mixture and composition of the latter, makes comparison of the results impossible.

III. THE INFLUENCE OF COPPER, MERCURY AND LEAD SALTS ON LIPOLYSIS.

In an earlier paper [Corran and Lewis, 1928] results were recorded on the influence of lead, both ionic and colloidal, on the lipolytic augmentation produced by serum. In every case increases in the effects due to serum were recorded. More recently the influence of various metallic salts has been investigated, generally in the absence of serum, but in some cases also in the presence of serum. 0.01 *M* aqueous solutions of cupric and mercuric chlorides and 0.01 *M* lead acetate were used. In all cases the duration of experiment was 48 hours and the temperature 37°.

Table V. *Influence of 0.01 M lead acetate on lipase in absence of serum.*

Lipase extract cc.	0.01 M Pb acetate cc.	Water cc.	Emulsion cc.	N/10 NaOH required cc.	Augmentation in N/10 NaOH cc.
1	0.00	5.00	5	25.70	—
1	0.25	4.75	5	26.10	+ 0.40
1	0.50	4.50	5	27.70	+ 2.00
1	1.00	4.00	5	30.90	+ 5.20
1	2.00	3.00	5	38.10	+ 12.40
1	5.00	0.00	5	40.10	+ 14.40

Table VI. *Influence of 0.01 M cupric chloride on lipase in absence of serum.*

Lipase extract cc.	0.01 M CuCl ₂ cc.	Water cc.	Emulsion cc.	N/10 NaOH required cc.	Augmentation in N/10 NaOH cc.
1	0.00	5.00	5	22.50	—
1	0.50	4.50	5	20.60	- 1.90
1	1.00	4.00	5	18.55	- 3.95
1	2.00	3.00	5	14.00	- 8.50
1	3.00	2.00	5	6.70	- 15.80
1	5.00	0.00	5	2.60	- 19.90

Table VII. *Influence of 0.01 M cupric chloride in presence of serum.*

Lipase extract cc.	Ox serum cc.	0.01 M CuCl ₂ cc.	Water cc.	Emulsion cc.	N/10 NaOH required cc.	Effect due to CuCl ₂ in N/10 NaOH cc.
1	0.0	0.00	5.00	5	22.00	—
1	0.5	0.00	4.50	5	44.00	—
1	0.5	0.25	4.25	5	41.10	- 2.90
1	0.5	0.50	4.00	5	34.60	- 9.40
1	0.5	1.00	3.50	5	29.50	- 14.50
1	0.5	2.00	2.50	5	26.50	- 17.50
1	0.5	4.50	0.00	5	6.50	- 37.50

Table VIII. *Influence of 0.01 M mercuric chloride on lipase in presence of serum.*

Lipase extract cc.	Ox serum cc.	0.01 M HgCl ₂ cc.	Water cc.	Emulsion cc.	N/10 NaOH required cc.	Effect due to HgCl ₂ in N/10 NaOH cc.
1	0.0	0.00	5.0	5	21.50	—
1	0.5	0.00	4.5	5	43.17	—
1	0.5	0.50	4.0	5	40.00	- 3.17
1	0.5	1.00	3.5	5	37.25	- 5.92
1	0.5	2.00	2.5	5	34.15	- 9.02
1	0.5	4.50	0.0	5	8.33	- 34.84

Copper exerts an inhibitory action on lipase in absence of serum. Moreover, both copper and mercury exert a similar effect on the co-enzymic activity of serum. On the other hand, lead exerts an augmentative effect on lipase, both in presence and in absence of serum. It might be of advantage to compare the augmentations produced by lead acetate in absence and in presence of serum. In absence of serum, 0.5 cc. of 0.01 M lead acetate solution gives an augmentation (in terms of N/10 NaOH) of 2.00 cc. In presence of serum¹ a similar quantity gives rise to an augmentation of 7.12 cc. Whether lead exerts an influence on the enzyme alone or whether it interacts, in addition, with the co-enzyme in serum, is not clear.

¹ These values are the minimum values recorded in an earlier paper [Corran and Lewis, 1928].

IV. THE LIPOLYTIC CO-ENZYME IN SERUM.

With the object of endeavouring to ascertain to which of the various constituents of serum the lipolytic co-enzyme may be attributed, and, if possible, of isolating the co-enzyme itself, ox-serum was submitted to a separation into its constituents.

Fresh ox-serum, previously centrifuged to remove any red blood-corpuscles, was employed. 50 cc. were dialysed in a collodion membrane, the distilled water in the surrounding vessel being changed every 6 hours. During dialysis, a precipitate was formed inside the membrane due to precipitation of a portion of the proteins in the serum. The dialysates were collected and thus the dialysable inorganic constituents of serum obtained. The mixture inside the membrane, consisting of serum freed from dialysable salts, was then filtered, the residue inside the filter-paper consisting of the euglobulin fraction. The filtrate was then acidified with a few drops of acetic acid and heated in a water-bath at 100°, when the proteins contained therein were precipitated. Thus the albumin + pseudoglobulin fraction was obtained, leaving in solution, after filtration, the lipins and their derivatives. No further separation was carried out. The fractions isolated, namely, inorganic compounds, euglobulin, pseudoglobulin + albumin, and lipins, were then made up to 50 cc. with water or, alternatively, evaporated to the required volume, *i.e.* so that the concentration of each constituent was identical with its concentration in the original serum, the protein fractions being in the form of coarse suspensions, which were shaken vigorously before volumes were measured out. Samples of 0.5 cc. of each mixture were taken and their lipolytic augmentations determined in the manner previously described. The accompanying table gives the results obtained.

Table IX.

Lipase extract cc.	Inorganic salts cc.	Euglobulin fraction cc.	Albumin + pseudo- globulin fraction cc.	Lipin fraction cc.	H ₂ O cc.	Emulsion cc.	N/10 NaOH required cc.
1	0.0	0.0	0.0	0.0	5.0	5	24.00
1	0.5	0.0	0.0	0.0	4.5	5	24.00
1	0.5	0.0	0.0	0.0	4.5	5	23.80
1	0.0	0.5	0.0	0.0	4.5	5	27.80
1	0.0	0.5	0.0	0.0	4.5	5	28.00
1	0.0	0.0	0.5	0.0	4.5	5	40.70
1	0.0	0.0	0.5	0.0	4.5	5	40.90
1	0.0	0.0	0.0	0.5	4.5	5	24.10
1	0.0	0.0	0.0	0.5	4.5	5	24.20

Table X. *Mean augmentations produced by each fraction of serum.*

	cc. N/10 NaOH
Inorganic salts	0.00
Euglobulin fraction	3.90
Albumin + pseudoglobulin fraction	16.80
Lipin fraction	0.15
Total	20.65 cc. N/10 NaOH

(0.5 cc. of untreated fresh ox-serum gives an augmentation of 22.0 cc. N/10 NaOH.) It is noteworthy that the total effects are as close together as they are in view of the somewhat drastic alterations in the physico-chemical state of the protein fractions.

It is evident that the albumin + pseudoglobulin fraction contains the greater part of the lipolytic co-enzyme. The value due to the euglobulin fraction may be due to the co-enzyme present in this fraction or possibly to incomplete separation of the albumin and euglobulin fractions. It is interesting to note that the inorganic salt fraction and the lipin fraction have no co-enzymic effect, thus bearing out the results recorded in a previous section of this paper.

SUMMARY.

(1) The influence of a number of different compounds on lipolysis has been investigated.

(2) The quantitative effects of purified lecithin and cholesterol were similar and only slight. In low concentration both compounds act as augmentors, the values of the augmentations subsequently falling with increasing concentration.

(3) The influences of sodium and potassium chlorides on lipase are only slightly augmentative. Calcium chloride, on the other hand, acts as an inhibitor, its quantitative effect being greater than that of sodium or potassium chloride.

(4) Ionic lead acts as an augmentor towards lipase in the presence and in the absence of serum. Copper, both in absence and in presence of serum, acts as an inhibitor, as does mercury.

(5) The albumin + pseudoglobulin fraction of blood-serum was found to possess over 80 % of the co-enzymic activity exhibited by serum.

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