LX. THE INFLUENCE OF NORMAL AND CANCEROUS BLOOD-SERUM ON PANCREATIC LIPASE ACTION AND THE EFFECT OF IONIC AND COLLOIDAL LEAD.

BY ROBERT FREDERICK CORRAN
AND WILLIAM CUDMORE McCULLAGH LEWIS¹.

*From the Muspratt Laboratory of Physical and Electro-Chemistry,
University of Liverpool.*

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THE "accelerating"² action of blood-serum and bile salts on pancreatic lipase has been observed by many authors (Pottevin [1903], Terroine [1914], Hamsik [1911,1915], Mellanby and Woolley [1914], and others). Perhaps the most notable contributions to the study of the action of various activators, of physiological importance, on lipase are those of Rosenheim and of Shaw-Mackenzie [Rosenheim, 1910; Rosenheim and Shaw-Mackenzie, 1910, 1, 2, 3; Shaw-Mackenzie, 1911, 1912, 1913, 1914], and as the methods and conclusions of these authors are closely connected with the work described in this paper, a very brief *résumé* of their conclusions is here given. Rosenheim [1910] found that the lipase contained in a glycerol extract of the pancreas could be separated into two portions, one portion consisting of inactive prolipase, insoluble in water and destroyed by heat, and the other consisting of a co-enzyme, soluble in water and thermostable. Bile salts and serum, according to Shaw-Mackenzie and Rosenheim, act as co-enzymes. They also found that a number of different compounds of varying structure can exert an augmentative action on lipase, for example, alcohol (in small quantity), sodium oleate, cholate and glycocholate, saponin and digitonin. Cholesterol, on the other hand, has a marked

¹ An investigation carried out on behalf of the Liverpool Medical Research Organisation; Prof. W. Blair Bell, director.

² "Acceleration" is the term apparently very generally employed in the literature of the subject, which deals with the influence exerted by various substances on the activity of lipase. Actually the greater part of the results recorded refer to changes in the *extent* of lipolysis effected, the values given being intended, in general, to represent final equilibrium values. Very frequently an increase in the extent of lipolysis is accompanied by an increase in the *rate* at the same time. But at least one case is recorded in the present paper where an increase in speed is accompanied by a reduction in the extent of lipolysis. Obviously the term "acceleration" ought to refer to alteration in rate, whilst the term "augmentation," i.e. augmentation of lipolysis, might be suggested as suitable for indicating an alteration in the extent (i.e. the final equilibrium values) of lipolysis. It is in this sense that the term "augmentation" is employed in the present paper.

diminishing action on the lipolytic augmentation of serum and bile salts. Concerning the nature of the co-enzyme of lipase present in serum no definite conclusions have been arrived at. Whereas cholesterol diminishes lipolytic action, lecithin derivatives augment it. Moreover, Willstätter [1923] has shown that simple salts of sodium and calcium can act as augmentors, consequently the inorganic salts play a part.

The results of Shaw-Mackenzie and Rosenheim show that in certain pathological conditions the augmentative action of serum is abnormal. They find that in human cases of scarlet fever and cancer the lipolytic augmentation is reduced and that, in cases of spontaneous recovery from cancer in mice, the augmentative action returns to normal. The results, however, do not appear to be conclusive, for in at least one paper [1910, 3] their results lead to the conclusion that cancer-serum augments to a much greater extent than does normal serum.

The work described in this paper was carried out to ascertain whether cancer-serum differed from normal serum with respect to its lipolytic co-enzyme content, and to study the effect of lead, in both the ionic and colloidal form, on the lipolytic augmentation produced by serum, both normal and cancerous.

Materials and method.

It was essential to use an enzyme extract which possessed the advantages of being reasonably pure and, at the same time, of being stable. An extract similar to that of Shaw-Mackenzie was employed. The method of preparation consisted of extraction of fresh, minced pig pancreas (which had been thoroughly washed with distilled water) with twice its weight of glycerol for 24 hours, and filtration of the mixture through a double layer of fine muslin. The turbid filtrate, containing lipase and also trypsin, proved to be a very satisfactory lipase extract in that the enzyme extract (kept in sterilised vessels in the ice-chest) was as active after a period of 9 months as at the beginning, thus differing from the enzyme extracts prepared by extraction with physiological salt solution [Amaki, 1926], by adsorption [Willstätter, 1923], or from pancreatic juice [Mellanby and Woolley, 1914], all of which were comparatively unstable.

As substrate an olive oil emulsion was used. The emulsions used by previous workers were generally prepared by neutralising the free fatty acid, present in the commercial oil, with standard sodium hydroxide, the soap formed acting as emulsifying agent.

As, however, different samples of olive oil contain varying amounts of free fatty acid, and consequently the composition of an emulsion prepared in the above manner varies, it was evident that an emulsion of pure olive oil with pure sodium oleate should be used to obtain a reliable substrate.

Pure olive oil was prepared by shaking the commercial oil with an equal volume of 10 % sodium hydroxide in a separating funnel at as high a temperature as the latter would stand (about 75°). After standing, the lower aqueous layer was separated and the emulsion of olive oil remaining washed with hot

distilled water until all soap was removed (in general about twelve washings were required). The olive oil was filtered through filter paper until all turbidity had disappeared and the oil dried finally by heating in an air-oven at 105° for 6 hours in a shallow basin. The oil was kept, pending use, in a sterilised flask fitted with a tight stopper in order to minimise the chance of production of free acid by bacterial action.

Pure sodium oleate was prepared by a modification of the method quoted by Leathes [1925]. Commercial olive oil was saponified with excess sodium hydroxide, the fatty acids were liberated by means of mineral acid and converted into lead salts by digestion on a water-bath with one-twentieth of their weight of litharge. Lead oleate was extracted with ether (the other lead soaps being insoluble in ether) and oleic acid liberated by the addition of dilute hydrochloric acid, the ether then being evaporated off. Neutralisation of the acid with standard aqueous sodium hydroxide in presence of alcohol gave a solution of sodium oleate, which could be adjusted, after removal of the alcohol, to the concentration required (1 % by weight).

In the experiments referred to hereafter, the enzyme extract, as described above, was used and an emulsion (prepared from four parts of pure olive oil and one part of 1 % sodium oleate solution) employed as substrate, except where otherwise stated. The emulsion used was fine grained, the size of the particles being on the average 5 μ .

The experimental procedure was similar in principle to that of Shaw-Mackenzie and Rosenheim, that is, it consisted of mixing the requisite quantities of lipase extract, serum, water (or aqueous solution) and oil emulsion and allowing them to react, at a temperature of 37°, until equilibrium was attained¹. The amount of hydrolysis was then determined by titrating the fatty acids produced against 0.1 *N* aqueous sodium hydroxide in presence of neutral alcohol (up to 40 % alcohol) and ether², using phenolphthalein as indicator.

I. EXPERIMENTS WITH COMMERCIAL OLIVE OIL EMULSIONS.

At the outset, measurements were carried out following the conditions of Shaw-Mackenzie, that is by using a commercial oil emulsion and normal ox serum, preserved under toluene. In general, the results obtained were similar to those obtained by him.

(a) *Effect of order of mixing of the reactants.*

Shaw-Mackenzie found that, if the enzyme and oil emulsion were mixed first and serum added last, no lipolytic augmentation by serum was observed, the augmentative action of serum only manifesting itself when serum was

¹ As the lipase extract possesses both lipolytic and liposynthetic properties, hydrolysis does not go to completion.

² Shaw-Mackenzie titrated in presence of alcohol only. Experiment showed that a more satisfactory end-point could be obtained by the addition of ether, *i.e.* following the mode of titration employed by Willstätter.

mixed with the enzyme and the emulsion added last. Experiments were therefore carried out to ascertain the effect of order of mixing of the reactants. In Tables I (a) and II (a) below, the enzyme and serum were mixed first, and the emulsion added last. In Tables I (b) and II (b) the enzyme and emulsion were mixed first, and the serum added last. The duration of the experiments is not arbitrary but corresponds to the attainment of equilibrium.

Table I (a).

Time of reaction, 18 hours. Temperature 37°.					
Lipase extract cc.	Ox serum cc.	Water cc.	Emulsion cc.	N/10 NaOH cc.	Augmentation N/10 NaOH cc.
1	0.0	5.0	5	1.15	—
1	0.5	4.5	5	18.97	17.82
1	1.0	4.0	5	27.45	26.30

Table I (b).

Lipase extract cc.	Emulsion cc.	Water cc.	Ox serum cc.	N/10 NaOH cc.	Augmentation N/10 NaOH cc.
1	5	5.0	0.0	1.00	—
1	5	4.5	0.5	20.27	19.27
1	5	4.0	1.0	16.48	15.48

Table II (a).

Glycerol extract diluted with twice its volume of water.

Lipase extract cc.	Ox serum cc.	Water cc.	Emulsion cc.	N/10 NaOH cc.	Augmentation N/10 NaOH cc.
1	0.0	5.0	3	0.33	—
1	0.5	4.5	3	10.89	10.56
1	1.0	4.0	3	17.86	17.53
1	2.0	3.0	3	19.20	18.87
1	3.0	2.0	3	20.75	20.42

Table II (b).

Lipase extract cc.	Emulsion cc.	Water cc.	Ox serum cc.	N/10 NaOH cc.	Augmentation N/10 NaOH cc.
1	3	5.0	0.0	0.30	—
1	3	4.5	0.5	15.50	15.20
1	3	4.0	1.0	19.70	19.40
1	3	3.0	2.0	16.95	16.65
1	3	2.0	3.0	16.45	16.15

It will be seen that in Tables I (a) and II (a) the effect of increasing concentration of serum is progressive up to a limit, but that in Tables I (b) and II (b) a maximum value is observed beyond which further addition of serum gives reduced values for the augmentative effect. It is of importance, therefore, that in carrying out comparative experiments, the reactants be mixed always in the same order. In the experiments described later, the serum and glycerol extract were mixed first, then the necessary quantity of water, or aqueous solution, and finally the oil emulsion. In the experiments with lead compounds, the serum was mixed first with the lead compound (a period of approximately

1 hour elapsing in order that equilibrium between the serum and the lead compound might be attained), then the glycerol extract of the enzyme was added followed by the water and the emulsion.

(b) *Effect of filtration of the glycerol extract on its lipolytic activity.*

Several workers have stated that, on filtration, the glycerol extract of lipase loses its activity. On the other hand, Hamsik found that filtration had no effect on the lipolytic activity of the enzyme extract. This point was investigated and it was found that filtration through asbestos or a Jena glass filter robbed the filtrate of its activity, the filter residues in both cases being highly active, but that filtration of the turbid extract through filter paper (type—Whatman No. 1) did not diminish the activity of the extract. Table III gives the results, using a turbid extract and a clear extract obtained by filtering the former through filter paper.

Table III

Lipase extract cc.	Ox serum cc.	Water cc.	Emulsion cc.	N/10 NaOH cc.	Augmentation N/10 NaOH cc.
Unfiltered turbid					
1	0	5	5	1.32	—
1	1	4	5	19.85	18.53
Clear paper-filtered					
1	0	5	5	1.38	—
1	1	4	5	20.89	19.51

It is probable, therefore, that the lipase occurs in the form of a finely divided suspension, rather than in the form of a coarse suspension.

II. EXPERIMENTS WITH PURE OLIVE OIL EMULSIONS.

Experiments were next carried out using pure olive oil and pure sodium oleate, and the effect of normal human serum was investigated on different glycerol extracts (marked *A*, *B*, and *C* in Table IV), in order to find out if

Table IV.

Time of reaction 48 hours. Temperature 37°.

	Lipase extract cc.	Normal serum cc.	Source of serum	Water cc.	Emulsion cc.	N/10 NaOH cc.	Augmentation N/10 NaOH cc.
<i>A</i>	1	0	—	5	5	20.58	—
	1	1	R. F. C.	4	5	41.00	20.42
<i>B</i>	1	0	—	5	5	22.42	—
	1	1	J. W.	4	5	43.28	20.86
	1	0	—	5	5	22.00	—
	1	1	E. H.	4	5	41.65	19.65
<i>C</i>	1	0.0	—	5.0	5	22.61	—
	1	0.5	S. K.	4.5	5	41.30	18.69
	1	0.0	—	5.0	5	21.60	—
	1	0.5	H. H.	4.5	5	39.44	17.84
	1	0.0	—	5.0	5	21.70	—
	1	0.5	L. T.	4.5	5	39.93	18.23

reproducible results could be obtained. In Table IV the augmentations produced by different samples of normal human serum are given using the different extracts. The initials in column 3 refer to the different people from whom the blood was obtained.

In the first place, it is apparent that the different samples are comparable with one another, and that the various samples of blood-serum yield very consistent values for their lipolytic augmentations. In the second place, the time of reaction was 48 hours, experiment showing that equilibrium had then been attained. The emulsion of pure olive oil was considerably less stable than the commercial emulsion of Shaw-Mackenzie and it was found necessary to shake the reaction vessels during the course of the present work at intervals of 10–12 hours in order to ensure the maintenance of complete emulsification of the oil¹. In the third place, the magnitudes of the control readings are considerably greater than those of Shaw-Mackenzie, who obtained large control readings in only a few cases. In many other cases, the control readings observed by Shaw-Mackenzie were less than 5 cc. As the principal uncontrolled variable in Shaw-Mackenzie's procedure was the amount of sodium oleate present, the influence of this salt on lipase action was investigated.

The rôle of sodium oleate in esterase and lipase action.

Three different substrates were used, (a) methyl acetate (a typical substrate for ester hydrolysis), (b) triacetin, as an example of a soluble glyceride, (c) pure olive oil. In the experiments with (a) and (b) a 10 % solution of the substrate in water was used. Tables V–VII give the mean results obtained on the influence of varying quantities of a 1 % aqueous solution of sodium oleate on esterase and lipase action. The values recorded are in all cases the mean of at least two (concordant) sets of experiments.

Table V.

Temperature 37°.					
Lipase extract cc.	Water cc.	1 % sodium oleate solution cc.	10 % aqueous methyl acetate cc.	Time hrs.	N/10 NaOH cc.
1	5.00	0.00	5	48	2.73
1	4.75	0.25	5	48	2.79
1	4.50	0.50	5	48	2.92
1	4.25	0.75	5	48	2.98
1	4.00	1.00	5	48	2.82
1	3.50	1.50	5	48	3.00
1	3.00	2.00	5	48	3.03
1	0.00	5.00	5	48	3.05

It is clear from Table V that the esterase content of the glycerol extract employed is very small, and no conclusions can be drawn from the results. On the hydrolysis of triacetin, sodium oleate exerts a marked augmentative action up to a definite concentration beyond which the augmentation dimin-

¹ This was tested from time to time by microscopic measurement of the size of the oil droplets.

ishes. The influence of sodium oleate on olive oil hydrolysis is remarkable in that, with increasing concentration of sodium oleate, a maximum degree of lipolysis, *i.e.* maximum augmentation, is reached beyond which the effect diminishes rapidly, so that in high concentration sodium oleate exerts a diminishing action on the *extent* of olive oil hydrolysis. On the other hand, sodium oleate, in increasing concentration, causes a progressively increasing acceleration of hydrolysis. In all the experiments recorded in Table VII, the system was repeatedly shaken. With low concentrations of oleate only temporary emulsification was obtained. With quantities of oleate greater than 1 cc. the emulsification was permanent.

Table VI.

Temperature 37°

Lipase extract cc.	Water cc.	1 % sodium oleate solution cc.	10 % aqueous triacetin cc.	Time hrs.	N/10 NaOH cc.	Augmentation N/10 NaOH cc.
1	5.0	0.0	10	48	8.86	—
1	4.5	0.5	10	48	9.95	1.09
1	4.0	1.0	10	48	10.34	1.48
1	3.0	2.0	10	48	10.69	1.83
1	0.0	5.0	10	48	9.70	0.84

Table VII.

Temperature 37°

Lipase extract cc.	Water cc.	1 % aqueous sodium oleate cc.	Olive oil cc.	Time days	N/10 NaOH cc.	Augmentation N/10 NaOH cc.
1	6.00	0.00	4	10	9.81	—
1	5.75	0.25	4	8	12.12	+ 2.31
1	5.50	0.50	4	6	14.98	+ 5.17
1	5.25	0.75	4	4	20.80	+ 10.99
1	5.00	1.00	4	3	22.68	+ 12.87
1	4.50	1.50	4	2	29.23	+ 19.42
1	4.00	2.00	4	2	35.80	+ 25.99
1	1.00	5.00	4	2	5.57	- 3.24
1	1.00	5.00*	4	2	2.80	- 7.01

* Of 5 % sodium oleate.

In Shaw-Mackenzie's experiments it was found that 5 cc. of commercial olive oil required, in general, 1–2 cc. of N/10 NaOH to neutralise the free acid present. Considering the two extreme values, namely 1 cc. and 2 cc. N/10 NaOH, calculation shows that 5 cc. of an olive oil emulsion, prepared by using 1 cc. and 2 cc. of N/10 NaOH respectively for neutralisation, contain the equivalent of 2.4 cc. and 4.3 cc. of 1 % aqueous sodium oleate, that is, a concentration region beyond the maximum value given in Table VII. Moreover, the greater the amount of free fatty acid contained in the commercial olive oil, the smaller will be the value of the control reading. It is probable therefore that variation in sodium oleate content is the chief cause of the variability observed in the control readings in Shaw-Mackenzie's results.

III. COMPARISON OF THE LIPOLYTIC AUGMENTATIONS OF NORMAL AND CANCEROUS SERUM.

The samples of blood were taken from the forearm and were, in all cases, centrifuged as soon as possible after extraction from the patient in order to avoid any change in the constitution of the serum. The reaction vessels consisted of thick-walled glass bottles of 100 cc. capacity, fitted with ground-glass stoppers; the bottles were subsequently used as titration vessels. The mode of following the reaction was the same as that described previously. In all the experiments carried out 1 cc. of enzyme extract was mixed with 0.5 cc. (or 1 cc.) of serum and the mixture made up to 6 cc. in every case by the addition of water. 5 cc. of the pure olive oil emulsion, previously mentioned, were then added and the reaction allowed to proceed. The following Tables VIII-X give the results obtained with normal and cancerous human serum. Under the column headed "source of serum" are given numerals, which serve to identify the different samples of serum used. In Table X a summary is given of the patients from whom blood was extracted, corresponding to the various numerals.

Table VIII.

Time of reaction 48 hours. Temperature 37°		
Amount of serum used cc.	Source of serum	Augmentation by serum N/10 NaOH cc.
1.0	Normal	20.42
1.0	I	18.34
1.0	Normal	20.86
1.0	II	14.10
1.0	III	14.88
1.0	Normal	19.65
1.0	VIII	12.30
0.5	Normal	17.56
0.5	IV	11.44
0.5	V	12.52
0.5	Normal	17.84
0.5	IX	17.24
0.5	X	11.20
0.5	Normal	18.64
0.5	VI	14.76
0.5	VII	12.53

Table IX.

Amount of serum used cc.	Source of serum	Augmentation by serum N/10 NaOH cc.
0.5	XII	12.93
0.5	XV	10.70
0.5	XVI	19.80
0.5	XVIII	15.30
0.5	XIX	10.64
0.5	XX	12.39
0.5	XXI	10.15
0.5	XXII	13.86
0.5	XXIII	13.60
0.5	XXIV	12.95
0.5	XXV	12.70

Table X.

Source of serum	Sex of patient	Initials	Nature of growth
I	Female	E. B.	Carcinoma cervix
II	Male	D. R.	Epithelioma tonsil
III	"	J. H.	"
IV	Female	L. L.	Carcinoma cervix
V	Male	J. W.	Carcinoma tongue
VI	"	J. M.	"
VII	"	G. W.	Epithelioma tongue
VIII	Female	E. W.	Carcinoma stomach
IX	"	E. C.	Sec. carcinoma chest
X	Male	J. B.	Epithelioma scrotum
XI	Female	E. S.	Carcinoma cervix
XV	"	J. I.	Rec. carcinoma cervix
XVI	Male	H. A.	Carcinoma oesophagus
XVIII	Female	A. J.	Rec. carcinoma chest
XIX	Male	E. W.	Epithelioma pharynx
XX	Female	A. G. M.	Carcinoma stomach
XXI	"	E. G.	Sec. carcinoma breast
XXII	"	S. D.	Carcinoma breast
XXIII	"	H. C.	Sec. carcinoma spine
XXIV	"	L. W.	Carcinoma cervix
XXV	"	M. J. B.	Post. nasal epithelioma

It is evident from the tables that there is a real abnormality in cancer-serum, in that it contains less of the lipolytic co-enzyme than does normal serum. This confirms the conclusions of Shaw-Mackenzie and Rosenheim. It will be observed that the deviations in some cases are greater than in others, even when the site of the tumour is the same. Inspection of Tables VIII-X demonstrates that in a few of the cases of malignancy investigated (cases I, IX, XVI), little difference exists in the augmentation by cancerous serum and normal serum. In fact, the sample of serum XVI augments lipase to a greater extent than does normal serum. It is significant that in this case the condition of malignancy was accompanied by secondary infections of the liver, infections of which are known to produce an excess of bile in the body, bile salts being active co-enzymes. In the other cases, however (I and IX), such secondary infections were not apparent.

It may be stated at this point that experiments were carried out in which the system was brought to a known initial p_H by means of a buffer (the ammonia-hydrochloric acid mixture of Michaelis and Willstätter) and the lipolytic augmentation of normal and cancerous serum investigated. The results obtained in these cases, though less exact owing to increased experimental difficulties (*e.g.* de-emulsification by the buffer), serve to bear out, in all respects, the conclusions arrived at above.

IV. THE EFFECT OF IONIC AND COLLOIDAL LEAD COMPOUNDS ON THE LIPOLYTIC AUGMENTATION OF SERUM.

Having established a definite variation in the lipolytic co-enzyme content of cancerous and normal serum, the effect of lead, in a variety of forms, on the augmentation of lipase by serum has been investigated. In this connection, the following lead compounds have been used: (a) saturated aqueous lead

phosphate, $\text{Pb}_3(\text{PO}_4)_2$; (b) 0.02 *N* lead acetate solution; (c) a finely divided lead suspension—"S₇"—(used locally as a therapeutic agent) containing 0.5 g. lead per 100 cc.; (d) a colloidal suspension of lead sulphide containing 0.2 g. lead per 100 cc.; (e) colloidal lead selenide containing 0.2 g. lead per 100 cc.

The serum was mixed first with the lead compound, the enzyme extract added, followed by water and lastly the emulsion. In the experiments recorded, the effect of lead on the augmentation produced by normal ox serum and cancerous human serum has been investigated. Tables XI–XIV give the results obtained using the various lead compounds mentioned. In all cases the temperature is 37° and the duration 48 hours.

Table XI. *The effect of lead on the lipolytic augmentation of ox serum.*

Lipase extract cc.	Serum cc.	H ₂ O cc.	0.02 <i>N</i> Pb acetate cc.	Saturated Pb phos- phate cc.	Lead suspen- sion S ₇ cc.	Colloidal PbSe cc.	Colloidal PbS cc.	Emulsion cc.	<i>N</i> /10 NaOH cc.
1	0.0	5.0	0	0	0	0	0	5	21.38
1	0.5	4.5	0	0	0	0	0	5	42.10
1	0.5	4.0	0.5	0	0	0	0	5	58.94
1	0.5	4.0	0	0.5	0	0	0	5	49.45
1	0.5	4.0	0	0	0.5	0	0	5	55.55
1	0.5	4.0	0	0	0	0.5	0	5	44.30
1	0.5	4.0	0	0	0	0	0.5	5	54.16

Table XII. *The effect of lead on human cancerous serum. Serum IV.*

Lipase xtract cc.	Serum cc.	H ₂ O cc.	0.02 <i>N</i> Pb acetate cc.	Saturated Pb phos- phate cc.	Lead suspen- sion S ₇ cc.	Colloidal PbSe cc.	Colloidal PbS cc.	Emulsion cc.	<i>N</i> /10 NaOH cc.
1	0.0	5.0	0	0	0	0	0	5	21.70
1	0.5	4.5	0	0	0	0	0	5	32.60
1	0.5	4.0	1	0	0	0	0	5	39.30
1	0.5	2.5	0	2	0	0	0	5	43.80
1	0.5	3.5	0	0	1	0	0	5	43.36
1	0.5	3.5	0	0	0	1	0	5	38.28
1	0.5	3.5	0	0	0	0	1	5	52.18

Table XIII. *Serum V.*

Lipase extract cc.	Serum cc.	H ₂ O cc.	0.02 <i>N</i> Pb acetate cc.	Saturated Pb phos- phate cc.	Lead suspen- sion S ₇ cc.	Colloidal PbSe cc.	Colloidal PbS cc.	Emulsion cc.	<i>N</i> /10 NaOH cc.
1	0.0	5.0	0	0	0	0	0	5	21.20
1	0.5	4.5	0	0	0	0	0	5	33.68
1	0.5	4.0	0.5	0	0	0	0	5	40.40
1	0.5	4.0	0	0.5	0	0	0	5	37.20
1	0.5	4.0	0	0	0.5	0	0	5	45.20
1	0.5	4.0	0	0	0	0.5	0	5	42.16
1	0.5	4.0	0	0	0	0	0.5	5	45.36

Table XIV. *Serum XV.*

Lipase extract cc.	Serum cc.	H ₂ O cc.	0.02 N Pb acetate cc.	Saturated Pb phos- phate cc.	Lead suspen- sion S ₇ cc.	Colloidal PbSe cc.	Colloidal PbS cc.	Emulsion cc.	N/10 NaOH cc.
1	0.0	5.0	0	0	0	0	0	5	21.40
1	0.5	4.5	0	0	0	0	0	5	32.45
1	0.5	4.0	0.5	0	0	0	0	5	39.57
1	0.5	4.0	0	0.5	0	0	0	5	36.00
1	0.5	4.0	0	0	0.5	0	0	5	41.00
1	0.5	4.0	0	0	0	0.5	0	5	34.95
1	0.5	4.0	0	0	0	0	0.5	5	37.50

It is evident from the preceding tables that lead, in both the ionic and colloidal forms, exerts a definite augmentative action on the lipolytic augmentation of serum. Although the influence of lead on the lipolytic augmentation of serum appears to be established, the magnitude of the action varies considerably from experiment to experiment. It may be pointed out, moreover, that these effects, although provisionally attributed to lead, might possibly not be specific to that metal; they may have their origin, in part at least, in the precipitation and removal from the field of some anti-enzymic material, possibly of a protein nature.

The effect of ionic and colloidal lead in low concentration.

In the experiments recorded above the concentrations of lead employed were high compared to that of the serum, the addition of the lead acetate solution, colloidal lead (S₇) and the suspension of lead sulphide causing precipitation of the greater part of the carbonate and phosphate; the lead selenide and lead phosphate solution cause no precipitation. The effect of lead in amounts insufficient to precipitate any of the constituents of serum is of special interest.

10 cc. of clear, fresh, ox serum were treated with 0.2 cc. of the various lead compounds employed in the last section for 6 hours. With the exception of lead selenide, it was found that clear mixtures remained, the dark colour of the lead suspensions having disappeared. In the case of the selenide, the dark colour of the latter remained after standing for 6 hours. 0.5 cc. of the mixture in each case was used. As a control 10 cc. of ox serum were mixed with 0.2 cc. of water and 0.5 cc. of the mixture used. The results obtained are given in Table XV.

It is clear that lead, in very low concentration, can increase the lipolytic augmentation produced by serum. Moreover, the results of Table XV lead to the conclusion that the effect of lead is due to interaction of lead with some constituent of the serum. It may be pointed out that the effect of lead is still apparent when the latter is present in quantity sufficient to precipitate the greater part of the phosphate and carbonate of the serum. It may also be mentioned that the augmentation obtained with the small quantities of lead

compounds (Table XV) are of the same order of magnitude as those obtained with much greater concentrations of lead. Thus, taking the case of saturated lead phosphate, the concentration in Table XV is one-fiftieth of that employed in Table XII, whilst the augmentation due to the smaller concentration is more than one-half that obtained with the greater concentration.

Table XV.

Lipase extract cc.	Serum cc.	Identification of serum	H ₂ O cc.	N/10 NaOH cc.	Augmentation N/10 NaOH cc.
1	0.0	—	5.0	20.00	—
1	0.5	<i>A</i>	4.5	41.00	21.00
1	0.5	<i>B</i>	4.5	46.30	26.30
1	0.5	<i>C</i>	4.5	43.90	23.90
1	0.5	<i>D</i>	4.5	48.30	28.30
1	0.5	<i>E</i>	4.5	42.70	22.70
1	0.5	<i>F</i>	4.5	48.30	28.30

A = mixture consisting of 10 cc. ox serum + 0.2 cc. H₂O

B = " " 10 cc. " + 0.2 cc. 0.02 *N* lead acetate

C = " " 10 cc. " + 0.2 cc. saturated lead phosphate

D = " " 10 cc. " + 0.2 cc. colloidal lead (*S*₇)

E = " " 10 cc. " + 0.2 cc. colloidal lead selenide

F = " " 10 cc. " + 0.2 cc. colloidal lead sulphide

DISCUSSION.

Considering the results collectively, it is clear that they afford a satisfactory confirmation of the conclusions arrived at by Shaw-Mackenzie and Rosenheim regarding the real augmentation of lipase action by normal serum and the reduced, but still positive, effect produced by the sera of cancer patients. The present data, it is believed, afford a more reliable basis for this conclusion than those given by Shaw-Mackenzie. The entirely new result is obtained that lead in a variety of forms—ionic and colloidal—when added to serum, either normal or cancerous, produces an increase in the augmentative effect. The presence of lead opposes and annuls the depression in lipolytic augmentation produced by cancerous serum as compared with normal serum.

Of the nature of the co-enzyme in serum we know as yet very little. Rosenheim has considered that it is hormonal in nature but existing evidence is insufficient to justify any definite conclusion.

SUMMARY.

1. The previous work on the influence of serum on pancreatic lipase is described briefly.

2. The method of preparation of the reactants is outlined. Experiments carried out using a commercial olive oil emulsion showed that (*a*) variation in the order of mixing of the reactants gave quantitatively different results for the effect of ox serum on lipase action, (*b*) filtration, through filter paper, has no effect on the activity of the glycerol extract of lipase.

3. A series of experiments has been carried out using purified olive oil and known quantities of sodium oleate as emulsifying agent. It has been found

that in concentrations up to 0.2 g. oleate per 100 cc. of the mixture, this salt acts, on the *extent* of fat hydrolysis, as an augmentor, whilst beyond this concentration the augmentative effect diminishes and with a concentration of 0.5 g. of oleate per 100 cc. mixture the effect is less than in the absence of the oleate. On the *rate* of hydrolysis, sodium oleate acts as an accelerator in all concentrations employed.

Sodium oleate likewise acts as an augmentor for the hydrolysis of triacetin, which forms a solution in water, the augmentation rising to a maximum at a concentration of 0.3 g. oleate per 100 cc. Thereafter, the augmentation diminishes slightly. It is concluded therefore that the oleate, as a co-enzyme, functions by interaction with the lipase and not with the surface of the substrate, although, when the latter is insoluble, the oleate is a highly efficient emulsifier.

4. Experiments on the comparative actions of normal and cancerous human serum on olive oil hydrolysis (*a*) in systems in which no attempt was made to control the initial p_H of the system, (*b*) in systems brought to a known initial p_H by means of a buffer, show that cancer-serum has a reduced augmentative effect on lipase, as compared with normal serum, thus confirming the conclusions of Shaw-Mackenzie.

5. The effect of ionic and colloidal lead on the lipolytic augmentation of serum has been investigated and it is found that lead, in both the ionic and colloidal forms, increases the lipolytic augmentation of both normal and cancerous sera. This effect manifests itself whether the lead compound is present in amounts either sufficient or insufficient to cause a precipitate with serum.

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