CLXXIX. INVESTIGATIONS IN ENZYME ACTION
DIRECTED TOWARDS THE STUDY OF THE
BIOCHEMISTRY OF CANCER.
THE ACTIVATION OF PANCREATIC PRO-LIPASE.

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THE marked increase in the lipolytic activity of extracts of fresh pancreas towards olive oil emulsion and similar substrates which is occasioned by the presence of a small proportion of blood-serum has been demonstrated by many workers. Differences in the augmenting effects of human sera have been utilised for diagnosing such abnormalities as the presence of malignant disease. The phenomenon of lipolytic augmentation was studied in particular by Rosenheim [1910; 1915; 1925] and by Shaw-Mackenzie [1911; 1912; 1913; 1914; 1931], who concluded that in many instances serum from patients suffering from progressive cancer activated the enzyme to a less extent than normal serum. More recently Corran and Lewis [1928] found that their experiments confirmed these findings.

Some preliminary work by Sanders at this laboratory [1932] also suggested that the augmenting effect of human serum was diminished in cancer and was subsequently raised by lead or radium treatment, although the change was not sufficiently regular for following the progress of the disease. On the other hand the augmenting effect of the serum of tumour-bearing mice appeared to be slightly raised.

The present paper describes experiments devised to enquire into certain problems connected with the mechanism of lipolytic activation and with the effects thereon of some organic and inorganic compounds of therapeutic interest, together with an examination of the activity of normal and cancerous blood-sera and other physiological fluids on a modified form of the enzyme material.

The increased activity of the enzyme in presence of serum is ascribed to the interaction of a thermostable co-enzyme in the blood. Corran [1929] showed that the agent is particularly associated with the albumin and pseudoglobulin of the serum and is absent from the salt and lipin fractions. A co-enzyme with similar activating properties is present in glycerol extracts of pancreas and its removal results in the complete inactivation of the preparation.

The production of such inactive pro-lipase was described by Rosenheim [1910] who showed it to be thermolabile and capable of being activated by tissue extracts and serum. The separation of the pancreatic pro-lipase and co-enzyme was effected by filtration or by precipitation of the pro-lipase by diluting the extract with 4 parts of water and separating the flocculent precipitate. This could be subsequently dried with alcohol and ether and used in the form of powder. Magnus [1904] had also separated the activator of lipolytic liver extract by dialysis.

In the present research a form of this pro-enzyme has been very extensively employed. The values for the activating effects of sera and other substances have been determined with this material, as it was thought that it would be an advance in the technique of the lipase test for cancer diagnosis to measure the activating power of serum on the inactive pro-lipase in the absence of the pancreatic co-enzyme. In this way the full augmenting, or, as it is preferably termed, the "activating power" of the serum is measured and the results should therefore be more definite and reliable.

EXPERIMENTAL.

A. METHODS.

1. *Technique of tests.* The tests were carried out in small (1 oz.) bottles fitted with rubber bungs. The lipase extracts were introduced by means of a micro-burette and the olive oil emulsion by means of a wide-bored pipette. The reagents were always added in the order (1) lipase extract, (2) serum, co-enzyme or other activator, (3) water, (4) olive oil emulsion, since Corran [1929] had found that the augmenting power of the serum was affected by the order in which the reagents were mixed, although in no case have we found the variation of lipolysis to be greater than could be ascribed to experimental error. Possibly the variations in the lipolysis found by previous workers were caused by the flocculation of the enzyme-protein complex on diluting the glycerol extract. This change, as shown in a later part of this paper, very readily occurs within certain limits of p_H . The mixtures were incubated for 48 hours at 37°, being vigorously shaken during the whole period in a special shaking machine which dealt with a total of 96 bottles in an incubator 12" × 14".

It had previously been determined that the interacting phases were in complete equilibrium after 48 hours' incubation under these conditions. The acid liberated (*i.e.* the degree of lipolysis) was determined by the usual method of titration to phenolphthalein with $N/10$ NaOH in presence of alcohol and ether.

2. *The enzyme material.* The following pancreatic extracts were employed in this work.

- (a) Glycerol extracts; 1 part pig gland: 2 parts glycerol.
- (b) Aqueous extracts; 1 part pig gland: 2 parts water.
- (c) Glycerol and aqueous extracts diluted 10 times with distilled water.

(a) *Glycerol extracts.* These were prepared from the minced glands exactly as described by previous workers. With these extracts it was found that duplicate tests often showed marked variations. Centrifuging the extracts for 3 minutes at 1500 r.p.m. somewhat improved the consistency of the results.

(b) *Aqueous extracts.* Much more consistent results were obtained by employing extracts prepared by shaking the minced gland with 2 parts of sterile distilled water for 2 hours, filtering the milky liquid through a fine mesh sieve and centrifuging for 3 minutes at 1500 r.p.m.

Contrary to the experience of some workers, we found that this extract, if prepared with aseptic precautions, kept cool (in refrigerator at 3–5°) and further preserved from bacterial action by the addition of a few drops of chloroform to each 100 cc., did not lose its activity for a period of many weeks.

Exp. 1. Stability of aqueous pancreatic extract.

0.5 cc. extract incubated for 48 hours with 4.0 cc. standard olive oil emulsion, prepared as described in section 3.

Age of extract	Acid liberated (cc. N/10 NaOH)
24 hours	31.8
3 weeks	31.4
8 "	30.0
10 "	25.2

(c) *Pro-lipase extract.* We were able to repeat the separation of the inactive pro-lipase from the glycerol extract of pancreas by Rosenheim's dilution technique and to confirm that it was reactivated by serum obtained from man and animals and also by tissue extracts including boiled pancreas extract¹.

Exp. 2. Test of pro-lipase as prepared by Rosenheim's method.

Glycerol extract of minced pig's pancreas 1 part gland: 2 parts glycerol; kept 48 hours; filtered through double muslin; 10 cc. run into 40 cc. distilled water in tall cylinder; mixed gently; kept 20 hours; deposit centrifuged; washed twice with 20 cc. water; made up to 10 cc. with 80 % glycerol.

Pro-lipase cc.	Activator cc.	Olive oil emulsion cc.	Titration cc. N/10 NaOH
0.5	—	5.0	1.2
0.5	1.0 co-enzyme*	5.0	19.6
0.5	0.5 ox serum	5.0	32.7
0.5	0.5 rabbit serum	5.0	25.6
0.5	0.5 human serum	5.0	30.8
—	1.0 co-enzyme*	5.0	0.85
—	0.5 ox serum	5.0	0.60

* Prepared by boiling equal volumes of aqueous pancreas extract and water and filtering.

Unfortunately we found much difficulty in obtaining sufficient quantities of the precipitate by this method. By the following slight modification, however, a definite and speedy flocculation was invariably obtained.

90 cc. of distilled water in a 100 cc. cylinder were acidified with exactly 0.1 cc. of pure lactic acid and 10 cc. of the aqueous extract were added. In about 6 hours the flocculated pro-lipase formed a compact sediment in the lower 10 cc., the supernatant liquid usually being quite clear. This was siphoned

¹ I am indebted to Dr Rosenheim for a private communication regarding his method.

away, the deposit of pro-lipase centrifuged and treated as described above. This simple technique was evolved after many unsuccessful efforts to procure a suitable pro-lipase by other methods, including alcohol or acetone precipitation, dialysis, precipitation by magnesium sulphate, *etc.*

The flocculated pro-lipase when washed and made up to a suitable volume was quite inactive towards olive oil emulsion but became definitely active in the presence of serum or of co-enzyme solutions prepared from glycerol or aqueous pancreas extracts by diluting them with an equal volume of water, boiling and filtering the coagulum from the clear fluid. If so desired, the precipitated pro-lipase could be washed with acetone and ether and thus obtained as a dry powder without appreciable loss of potency.

Exp. 3. Acid-flocculated pro-lipase.

Pro-lipase	Activator cc.	Olive oil emulsion cc.	Titration cc. <i>N</i> /10 NaOH
0.5 cc. aqueous emulsion	—	4.0	2.0
0.5 " "	1.0 co-enzyme	4.0	28.5
0.5 " "	0.5 ox serum	4.0	35.3
approx. 0.5 mg. powder	—	4.0	1.4
" 0.5 "	1.0 co-enzyme	4.0	25.0
" 0.5 "	0.5 ox serum	4.0	28.3

(*d*) *Diluted extracts.* Preliminary experiments carried out to determine the optimum quantities of the reagents for the lipase tests demonstrated that when much smaller quantities of the ordinary lipase extract than hitherto utilised were employed in conjunction with the usual volume of olive oil, a considerable amount of lipolysis still ensued. Thus, when only 0.05 cc. of either glycerol or aqueous extract was used in conjunction with 0.5 cc. of ox serum the titration obtained was approximately the same as when the full 0.5 cc. was used.

Exp. 4. Effect of diminishing amount of lipase extract.

Lipase cc.	Activator cc.	Olive oil emulsion cc.	Titration cc. <i>N</i> /10 NaOH
0.5 aqueous	—	4.0	40.7
0.1 "	—	4.0	19.3
0.05 "	—	4.0	1.8
0.05 "	0.5 ox serum	4.0	41.8
0.5 glycerol	—	4.0	35.2
0.05 "	—	4.0	2.0
0.05 "	0.5 ox serum	4.0	33.5

It will be noted that the extent of lipolysis does not vary directly with the quantity of lipase added; below a critical minimum amount the reaction is practically zero. This suggests that a certain concentration on the surface of the droplets of oil is needed to enable the reaction to proceed; the addition of co-enzyme may enable the orientation of the colloidal particles to take place with small amounts of the actual pro-enzyme material. In a similar fashion Willstätter [see Waldschmidt-Leitz, 1929, p. 65] has suggested that the effect of proteins and salts such as calcium oleate in augmenting lipase action is

dependent upon the formation of complexes which bring the enzyme into more intimate contact with the substrate.

Experiments with diluted glycerol and aqueous lipase were then made which showed that 0.5 cc. of either extract, previously diluted 10 times with glycerol or water respectively, was able, in the presence of 0.5 cc. of serum, to hydrolyse to the same extent as 0.5 cc. of the original extract.

Exp. 5. Comparison of undiluted and diluted extracts.

G. = Glycerol extract.		A. = Aqueous extract.		
Lipase diluted cc.	Lipase undiluted cc.	Activator cc.	Olive oil emulsion cc.	Titration cc. N/10 NaOH
—	0.5 G.	—	4.0	29.6
—	0.5 A.	—	4.0	25.7
0.5 G.	—	—	4.0	1.4
0.5 G.	—	1.0 co-enzyme	4.0	20.9
0.5 G.	—	0.5 ox serum	4.0	29.3
0.5 A.	—	—	4.0	0.95
0.5 A.	—	1.0 co-enzyme	4.0	17.3
0.5 A.	—	0.5 ox serum	4.0	26.6

This demonstrates that the glycerol and aqueous extracts contain a great excess of pro-enzyme over the activator, a fact which suggests that the part played by the tissue juices and the blood-serum in activating the inert enzyme material (in which form it largely exists in the gland), is of the utmost importance in fat metabolism in the body.

By diluting these solutions sufficiently the proportion of co-enzyme can be reduced to negligible amounts—the diluted enzyme solution will therefore be inactive but will contain a relatively large amount of latent pro-enzyme which can readily be activated by adding *e.g.* 1 cc. co-enzyme solution or 0.5 cc. serum, in which case titrations of 20–30 cc. N/10 NaOH are obtained after 48 hours' incubation with 4.0 cc. olive oil emulsion. It was observed that ox serum was invariably more effective in activating the dilute extracts than an equal volume of co-enzyme; 0.3 cc. of serum was usually able to activate to its full extent 0.05 cc. of undiluted extract or 0.5 cc. of diluted solution.

Exp. 6. Activation value of ox serum for diluted extracts.

Lipase diluted extract cc.	Activator cc.	Olive oil emulsion cc.	Titration cc. N/10 NaOH
0.5	—	4.0	1.2
0.5	0.1 ox serum	4.0	3.6
0.5	0.2 „	4.0	14.5
0.5	0.3 „	4.0	32.8
0.5	0.4 „	4.0	31.6
0.5	0.5 „	4.0	32.0
0.5	1.0 co-enzyme	4.0	18.0
0.5	—	—	—

It will be seen from these figures that the diluted extract can be utilised for certain experiments in which pro-lipase free from co-enzyme is required, thereby saving the time necessary for the isolation of the pro-enzyme by the flocculation methods.

The inactive material in both forms has been found very suitable for estimating the activation value of sera and other fluids; the figures obtained are a direct measure of the activity bestowed on the previously inert material.

The validity of this statement was proved by experiments in which varying quantities of pro-lipase were activated by a constant volume of serum. When the co-enzyme value of the serum was fully exerted it was not possible to cause a greater lipolysis by increasing the content of pro-lipase, *i.e.* the reaction was limited by the co-enzyme available.

Exp. 7.

Pro-lipase obtained by sedimentation from 20 cc. of aqueous extract of pancreas in presence of 0.1 % lactic acid; centrifuged and washed with water 3 times; volume made up to 20 cc. with distilled water. Action on olive oil emulsion tested after incubation for 48 hours at 37°.

Pro-lipase cc.	Serum cc.	Water cc.	Emulsion cc.	Titration cc. N/10 NaOH
0.5	—	1.5	4.0	1.1
1.0	—	1.0	4.0	1.4
0.25	0.5	1.75	4.0	37.8
0.5	0.5	1.5	4.0	38.8
0.75	0.5	1.25	4.0	38.0
1.0	0.5	1.0	4.0	36.4
0.5	0.2	1.8	4.0	30.3

3. *The olive oil emulsion.* The oil was purified with sodium hydroxide as recommended by previous workers. The sodium oleate was prepared by neutralising pure oleic acid. In the method used for routine tests an emulsion was prepared by adding 3.75 cc. of 7.5 % sodium oleate to 100 cc. of purified olive oil, instead of 25 cc. of 1 % as used by other workers. This gave a stable emulsion and reduced the quantity of aqueous phase to a minimum, a condition which was desired for some of the tests. Various experiments proved that this proportion of sodium oleate emulsified the oil suitably and gave a reliable substrate.

Exp. 8. Effect of sodium oleate.

Lipase (aqueous) cc.	Serum cc.	Water cc.	Emulsion cc.	Olive oil cc.	Titration cc. N/10 NaOH
0.5	—	1.0	—	4.0	28.6
0.5	—	1.0	4.0	—	36.1
0.5 (diluted by 10)	0.5	0.5	—	4.0	27.2
0.5 „	0.5	0.5	4.0	—	33.0

4. *Quantities of reagents.* For most of the preliminary work the following volumes of reacting fluids were found to be very suitable:

	cc.
Lipase extract	0.5
Serum	0.5
Co-enzyme or other activating solution	1.0
Olive oil emulsion	4.0
Water to bring total volume to	6.5

For quantitative tests on sera this method was afterwards modified by reducing the volume of serum to 0.2 cc. in accordance with the results of the

following experiment in which the activation values of varying quantities of ox serum towards the pro-lipase were determined.

Exp. 9. Activating values of varying amounts of ox serum towards pro-lipase.

1.0 cc. samples of pro-lipase, inactive towards olive oil emulsion, were distributed into a series of bottles; amounts of ox serum from 0.05 to 1.0 cc. added; incubated 48 hours at 37° with 4 cc. olive oil emulsion.

Serum (cc.)	Titration cc. N/10 NaOH
0.00	2.0
0.05	11.0
0.075	16.5
0.10	25.6
0.15	31.4
0.20	34.5
0.25	36.3
0.30	37.3
0.40	38.4
0.50	38.3
0.75	38.7
1.00	37.8

It will be observed that the addition of serum up to 0.3 cc. results in increasing values in the acid liberated, but beyond this, very little alteration in the lipolytic value is brought about.

This result was confirmed by several tests using different samples of pancreatic lipase and serum. The general character of the reaction is also indicated by *Exp. 6*.

It is to be concluded therefore that for comparison of the activation values, the addition of large volumes of serum is not indicated.

B. RESULTS OF EXPERIMENTS.

1. *Action of some colloidal substances on pro-lipase.*

As demonstrated above, sodium oleate has the power of augmenting the action of lipase solutions in presence or absence of serum and of diluted lipase extracts in the presence of serum. This action may be due to (1) possible activation of the pro-lipase, (2) stabilisation of the olive oil emulsion.

By testing with diluted lipase extracts and precipitated pro-lipase it was found that the augmenting action must be ascribed to the influence on the substrate. Such extracts, in presence of 1 cc. of 1 % sodium oleate, remained quite inactive. Many other organic and inorganic colloidal substances were also tested for possible power towards pro-lipase.

It was found that while 2 % gelatin, 1 % gum arabic, 1 % gum tragacanth, 1 % agar were able definitely to augment the action of lipase on olive oil, none was able to activate the pro-lipase. The following series of compounds was tested and found to have no such activating power: starch (1 % suspension, or 0.5 % solution of Lintner's soluble starch), caseinogen, haemoglobin from ox blood corpuscles, colloidal zinc sulphide, suspension of powdered charcoal.

On the other hand a solution of raw egg-white in 5 times its volume of distilled water and a solution of "colloidal iron" (as supplied for protein precipitation for MacLean's method of estimation of blood-sugar) had a distinct effect in activating the pro-lipase.

The results are set out in Table I.

Table I. *Action of colloids on pro-lipase.*

Lipase cc.	Augmentor cc.	Water cc.	Emulsion cc.	Titration cc. N/10 NaOH
0.5 aqueous dil.	—	0.5	4.0	1.4
0.5 "	1.0 gum arabic	0.5	4.0	1.3
0.5 "	1.0 egg-white	0.5	4.0	23.0
0.5 "	1.0 gum tragacanth	0.5	4.0	2.3
0.5 "	1.0 "colloidal iron"	0.5	4.0	29.0
0.5 "	1.0 egg-albumin	0.5	4.0	2.0
0.5 pro-lipase	1.0 egg-white	0.5	4.0	23.6
0.5 "	1.0 "colloidal iron"	0.5	4.0	10.0

It is not improbable that the activity of the egg-white was due to some unknown substance adsorbed on the proteins of the egg-white, for it was found that a sample of egg-albumin was without this action. Perhaps the activator present is utilised in the metabolism of the fat and lipins of the egg-yolk during the development of the embryo. In order to test whether the action of "colloidal iron" is associated with its potential oxidising capacities, tests were carried out with dilute solutions of hydrogen peroxide 1/4000, ferric chloride 0.1 % and N/400 potassium permanganate. None of these had any action in activating pro-lipase.

2. *Action of inorganic compounds on pro-lipase.*

It had been found by Corran and Lewis [1928] that colloidal lead and lead salts, *e.g.* lead acetate, were capable of augmenting lipase action. It was not demonstrated, however, whether this was the result of some reaction with the blood-serum, with the oil emulsion or with one or both of the constituents of the lipase complex.

We have found that some lead salts have a definite activating effect on pro-lipase. In this they contrast with various other heavy metal salts *etc.*, which were tested in various concentrations, *e.g.* cuprous acetate, cupric chloride, thallium acetate, uranium acetate, selenious acid, cadmium chloride, thus confirming Corran and Lewis [1928], who found that copper and mercury salts had an inhibiting effect on lipase in absence of serum and in presence of serum or co-enzyme (Table II).

The experiments show that the sum of the separate activating effects of lead *plus* serum is somewhat greater than their combined augmenting action.

It is of interest that Cooper and Nicholas [1930] obtained somewhat different results using castor oil seed (lipase liberated by dilute acetic acid). In their experiments lead acetate or chloride in dilutions from 1/5000 to 1/5,000,000 had no effect on the reaction, mercuric chloride and copper acetate inhibited, whilst

Table II.

Pro-lipase cc.	Ox serum cc.	Salt 1 % solution cc.	Water cc.	Olive oil emulsion cc.	Titration cc. N/10 NaOH
—	—	1.0 lead acetate	1.0	4.0	0.7
0.5	—	—	1.5	4.0	1.2
0.5	0.5	—	1.0	4.0	26.8
0.5	0.5	1.0 lead acetate	0.5	4.0	33.4
0.5	—	1.0 "	0.5	4.0	13.4
0.5	—	1.0 lead chloride	0.5	4.0	12.2
0.5	—	1.0 cuprous chloride	0.5	4.0	0.5
0.5	—	0.5 thallium acetate	1.0	4.0	0.5
0.5	—	1.0 uranyl acetate	0.5	4.0	1.8
0.5	—	1.0 cupric chloride	0.5	4.0	1.4
0.5	—	1.0 Co(NO ₃) ₂	0.5	4.0	14.8
0.5	—	1.0 MnCl ₂	0.5	4.0	3.0

lead glycine, lead triethyl chloride, thallium acetate or sulphate accelerated the lipolysis.

Thus it must be concluded either that the lipase is different from pancreatic lipase or that the reaction is affected by the substrate. In a succeeding portion of this paper it will be shown that such a change of substrate may materially alter the lipolytic action of the enzyme. On the other hand, evidence that the lipases in different organs have different characters has been given by many workers and is also afforded by such differences in physical constants as the following [Waldschmidt-Leitz, 1929].

	Pancreatic lipase	Ricin lipase	Gastric lipase
Opt. p_H	8.0	4.7	4.5
Temp. coeff. of reaction (20–30°)	1.26	1.23	—

3. Action of salts of organic acids.

The augmenting effect of sodium oleate was compared with that of a variety of organic salts, in order to ascertain to what degree this action (which had been found to be exerted through the substrate) was possessed by salts of various organic acids. Some of the unsaturated dibasic acids have been stated to play a definite rôle in the lysis of cancer cells [Freund and Kaminer, 1923] and might possibly do this by affecting lipolytic processes in these circumstances. It has also been stated that injections of oleic and linoleic acids and the sodium salts of these acids into mice caused an immunising action towards grafted tumours [Nakahara, 1924; 1925]. Since sodium oleate and stearate possess special properties of forming large micelles and colloid complexes, and thus differ materially from solutions of such salts as sodium acetate or sodium lactate, it would be expected that marked divergencies would be observed in their effects on the substrate and thus on the lipolytic action.

The following acids were tested, 1 % solutions of the sodium salts being utilised in all cases: lactic, citric, caproic, acetic, *n*-butyric, aminoacetic, maleic, tartaric, pyruvic, glycollic, malic, tiglic, ricinoleic, palmitic, stearic, sebacic, taurocholic, linoleic and glycerophosphoric.

In the first place none of these salts had any effect in activating pro-lipase, except taurocholate. This was a specimen of crude sodium taurocholate and caused a definite augmentation both with diluted aqueous extract and with flocculated pro-lipase (Table III *a*).

A number of these tests are illustrated in Table III *a*.

Table III *a*. *Effect of sodium salts of organic acids on inactive pro-lipase.*

(1 cc. of 1 % solution of salt for each test.)

Pro-lipase cc.	Organic salt	Water cc.	Olive oil emulsion cc.	Titration cc. N/10 NaOH
0.5 dil. extract	—	1.5	4.0	1.2
0.5 „	Na oleate	0.5	4.0	1.3
0.5 „	Na palmitate	0.5	4.0	0.8
0.5 „	Na citrate	0.5	4.0	2.0
0.5 „	Na taurocholate	0.5	4.0	7.8
1.0 pro-lipase	Na taurocholate	0.5	4.0	11.6
1.0 „	—	1.5	4.0	1.3

Many of these salts were capable, however, of augmenting the action of aqueous pancreatic lipase already lipolytic by reason of the presence of the natural co-enzyme. This is illustrated in Table III *b*.

Table III *b*.

Organic salts 1 % solution	Titration cc. N/10 NaOH
Control	38.0
Sodium oleate	47.3
„ acetate	50.4
„ citrate	53.6
„ lactate	51.1
„ ricinoleate	41.1
„ palmitate	42.2
„ stearate	38.6
„ linoleate	42.4
„ sebacate	48.8

The solutions were prepared from samples of pure B.D.H. acids and in all cases were adjusted so that they were “neutral” to phenolphthalein in presence of a mixture of alcohol and ether. No marked change was observed when, in a number of instances, potassium or calcium salts were substituted for the sodium salts.

Table III *c*. *Effect of sodium oleate on lipolysis of olive oil in presence of serum.*

Aqueous lipase cc.	1 % Na oleate cc.	Olive oil cc.	Ox serum cc.	Titration cc. N/10 NaOH
0.5	—	4.0	—	38.0
0.5	1.0	4.0	—	47.7
0.5	—	4.0	0.5	54.3
0.5	1.0	4.0	0.5	58.2

Since many of these organic acid salts caused an augmentation in lipolysis as great as that of sodium oleate it appears that their effects must be ascribed to the influence on the olive oil, *e.g.* by stabilisation of the emulsion.

Experiments carried out with a view to testing whether sodium oleate or other organic acid salts had, in addition, any direct effect on the activator of serum were inconclusive; the serum itself appears to help the emulsification of the oil very materially. This effect is indicated in Table III *c*.

4. *The action of pancreatic lipase and pro-lipase on neutral animal and vegetable oils.*

Olive oil forms an admirable substrate for testing the lipolytic activity of extracts, especially when purified and emulsified as described; it was thought to be worth investigating, however, whether various other natural fats and oils of similar composition behaved similarly towards the enzyme material. Samples of good quality oils, before and after treatment to remove traces of acid, were tested to compare their responses to the lipase. Table IV shows the results employing a potent aqueous extract, 0.5 cc. of enzyme, 4.0 cc. oil and 1.5 cc. water being used in all tests, incubation being for 48 hours.

Table IV. *Lipolysis of various oils by aqueous pancreatic lipase.*

Oil	Lipolysis—cc. N/10 NaOH	
	Crude	Purified
Coco-nut	71.8	66.8
Linseed	64.8	30.3
Castor	60.6	62.4
Palm	55.6	41.2
Hog lard	53.5	52.9
Cottonseed	50.6	44.4
Olive oil emulsion	46.8	—
Croton	45.0	28.2
Olive	40.8	48.0
Rape	41.0	41.2
Neatsfoot	38.6	40.4
Cod-liver	34.0	6.9
Chaulmoogra	25.2	58.0

It is evident that most of the above fats and oils are very sensitive to the action of lipase, many giving higher values of liberated acid than olive oil. The chief exceptions were cod-liver oil and crude chaulmoogra oil. The latter was somewhat acid and much greater lipolysis was obtained on using the purified material.

The low value with cod-liver oil which was invariably obtained is remarkable. It should be mentioned that this oil, even when treated with alkali, had a high content of cholesterol.

Experiments were made to test the effect of such dissolved cholesterol by making solutions of 1 % and 5 % pure cholesterol in warm olive oil. It was found that these solutions were not acted upon by pro-lipase. Also no lipolysis by pro-lipase was occasioned by the presence of 1 cc. of a cholesterol suspension (produced by dropping a 1 % alcoholic solution into water) when either olive oil or olive oil emulsion was used as substrate.

With glycerol lipase extracts Corran [1929] found that a coarse suspension of cholesterol acted as an augmentor. We also found that our colloidal suspension behaved as an augmentor to aqueous lipase extracts acting on olive oil emulsion. The low value of the lipolysis with the cod-liver oil is not attributable therefore to the presence of this impurity.

It is of interest that Ezoe [1929] found that the enzyme in spleen extracts digested oils in the following order: olive, sesame, rape, peanut, linseed, palm, whale, lard, castor, sardine, walnut, tripalmitin. This differs markedly from the order obtained with the pancreatic extract.

The action of pro-lipase, in presence and in absence of activator in the form of blood-serum was also tested on this series of fatty oils.

It was found that some of the unpurified oils were hydrolysed by the pro-lipase in absence of serum, suggesting that they contained activator themselves. It will be observed from Table V, that whereas the pro-lipase was inert towards hog lard, cod-liver oil, olive oil, croton oil and rape oil, a distinct reaction resulted with chaulmoogra, palm, coco-nut, neatsfoot, linseed and cottonseed oils.

Table V. *Lipolysis of oils by pro-lipase in presence and absence of activator.*

0.5 cc. pro-lipase emulsion. 4.0 cc. oil. 0.5 cc. ox serum.

Incubated for 48 hours with shaking.

Oil	Titration cc. <i>N</i> /10 NaOH	
	Serum present	Serum absent
Lard	44.5 (33.2)	2.2 (3.0)
Cod-liver	39.7 (17.8)	3.5 (2.4)
Croton	35.7 (21.7)	4.0 (2.6)
Olive	46.8 (42.8)	5.4 (5.0)
Rape	38.3 (42.5)	6.3 (5.2)
Neatsfoot	46.4 (38.4)	32.5 (3.9)
Chaulmoogra	31.0 (38.9)	27.3 (24.7)
Palm	45.2 (49.3)	27.2 (26.3)
Coco-nut	66.2 (62.5)	56.0 (23.4)
Linseed	50.0 (45.5)	45.0 (18.5)
Cottonseed	47.7 (39.7)	38.7 (10.1)
Castor	46.9 (36.6)	31.3 (29.3)
Human "depôt fat"	43.6	32.5

The figures in brackets were obtained after washing the oils with 1 % NaOH and then with hot water until the washings were neutral to phenolphthalein.

In some instances the washing apparently removed an appreciable amount of some activating substance, but in the cases of castor and chaulmoogra oils little change in the lipolysis ensued. Either, therefore, these oils retain the activator with very great tenacity or we have the remarkable instance of a form of lipase which is quite inert towards one group of neutral oils yet definitely active towards another series.

This possibility is of interest in connection with tumour formation since certain disturbances in metabolism might result in the formation of types of fats which would be more liable to hydrolysis and resynthesis than normal animal fats, thus causing further disorder in cell and tissue economy.

Ovarian dermoid cysts have been found to contain constituents such as cetyl or citosyl alcohol and cholesterol isomerides foreign to normal tissues. Also it appears that in some cases of malignant tumours the fats of the neoplasm are not available for general metabolism. Hirsch and Wells [1920] described a case in which a liposarcoma weighing 69 lbs. and containing 2 lbs. of fat was removed from a body in which normal fat depôts were completely exhausted.

5. *The activation of pro-lipase by physiological fluids and extracts.*

(a) *Heat-coagulated serum-protein.* By precipitating the proteins by heat-coagulation Corran [1929] found that the albumin and pseudoglobulin fractions of serum contained 80 % of the co-enzyme activity of blood-serum and that the dialysate was inactive. By first diluting blood-serum with 5 volumes of water, bringing to the isoelectric point with dilute acetic acid and then precipitating the proteins by heat we observed that the clear fluid obtained after removing the clotted proteins and concentrating had definite activating properties (Table VI, fluid 1). A portion of this fluid was dialysed against running water

Table VI. *Activation of pro-lipase by serum-proteins and physiological fluids.*

Specimen	Activation value
	cc. N/10 NaOH
Serum fluid 1	31.3
" " 2	17.1
" dialysate	12.9
" fluid 3	16.3
Alcohol-precipitated protein	26.0
Normal urine	24.1
" cerebro-spinal fluid	21.2
Ovarian fluid	26.3

for 36 hours after which it was found to have decreased in activating power by 50 % (Table VI, fluid 2). By dialysing a further portion against 500 cc. of distilled water for 24 hours and concentrating the dialysate to 20 cc. the passage of the activator through the membrane was also demonstrated (Table VI).

(b) *Alcohol-precipitated proteins.* The precipitate obtained by running 5 cc. of serum into 50 cc. of 50 % alcohol was washed with distilled water and made into emulsion with 20 cc. of water and tested for activator. The alcoholic fluid which was removed was concentrated to 10 cc. and also tested (Table VI, fluid 3). Both fractions gave a positive result.

(c) *Urine, cerebro-spinal fluid and ovarian fluid.* The activation values for specimens of urine, cerebro-spinal fluid and ovarian fluid are also given in Table VI. They were all potent activators; none possessed any intrinsic lipolytic activity. The urine and cerebro-spinal fluid were normal samples while the ovarian liquor was obtained from a cystic ovary removed at an operation and kindly supplied by Prof. Beckwith Whitehouse.

6. *Activation of normal and cancerous human blood-sera.*

Tables VII and VIII give the activation values obtained in a series of normal human sera and in sera from cancer patients respectively, using the quantities of reagents described previously. The normal sera gave high values (30–40 cc. *N*/10 NaOH) for the activation. It will be noted that the figures for

Table VII. *Activation values of normal sera.*

Blood sample	cc. <i>N</i> /10 NaOH
1	37.0
2	39.9
3	39.6
4	39.0
5	38.0
6	35.6
7	40.7
8	39.3
9	43.0
10	37.3
11	38.8
12	31.8
13	36.3
14	35.6
15	34.1
16	38.0
17	38.8
18	36.3
Mean	37.7

Table VIII. *Activation values of sera from cancer patients.*

Blood sample	cc. <i>N</i> /10 NaOH
1	38.8
2	31.8
3	36.3
4	30.6
5	31.6
6	37.3
7	38.0
8	35.9
9	39.4
10	41.3
11	40.6
12	31.8
13	34.8
14	34.9
15	35.8
16	36.8
17	37.2
18	39.8
19	35.1
20	35.3
21	36.6
22	35.1
23	33.5
24	33.0
Mean	35.5

the cancer sera are also consistently high and no very marked differences are to be observed between the individual figures or the average values. The majority of these cancer sera were obtained from pre-operative cases and from

a variety of types of malignant disease, diagnosis being made by members of the Honorary Staff of the Birmingham General Hospital.

The examination of a considerable number of specimens has now been carried out and the results confirm those indicated in the series quoted; the values obtained show that no specific alteration in the activation value of the blood-serum for pro-lipase occurs in cancerous conditions.

Certain observations made during the course of the investigation suggest, however, that it would be of value to examine the effect of lead and other metallic salts on the pro-lipase content of normal and tumour tissue.

SUMMARY AND CONCLUSIONS.

For the purpose of investigating the properties of normal and cancerous serum in respect of their activating powers towards lipolytic pancreas extracts, a method has been elaborated for the separation of the inactive pro-lipase fraction of pancreas in sufficient quantities for the experimental examination of its interaction with chemical compounds, and with serum-co-enzyme and other physiological fluids.

A quantitative comparison of the lipolysis of various animal and vegetable fats by aqueous pancreatic extract and by this special form of flocculated pro-lipase has been carried out. Whereas pro-lipase is inert towards cod-liver oil, olive oil, hog lard and croton oil it readily decomposes palm oil, chaulmoogra and coco-nut oils even when these have been purified.

A co-enzyme capable of activating the pro-lipase is present in urine and in ovarian fluid.

Very small amounts of human and animal serum are capable of activating pro-lipase to the maximum extent. According to the results with a large number of sera obtained from varied types of malignant disease, and contrary to the opinion of some previous workers, the serum of cancer patients was just as efficient in activating the lipase as that from normal persons.

It has been observed that certain lead salts are able to activate the pro-lipase to an appreciable extent. This property is almost unique among the metallic compounds tested. The observations made in this investigation suggest that an examination of the pro-lipase content of tumours and the influence of lead and other metallic salts thereon would be of interest.

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