XXVIII. ON THE LIPOLYTIC ACTION OF THE BLOOD.

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Our knowledge of the ultimate processes involved in the storage of fat and its mode of transport from the fat depots to the tissues requiring it is very scanty. The fate of fat from the intestine to its presence in the thoracic duct is accurately known from the experiments of Moore [1897]. From the thoracic duct it passes into the blood and is rapidly converted into a substance no longer extractable by ether, which can pass through cell membranes to be stored up as fat in the fat depots or to be used as a source of energy. Of the nature of this substance our knowledge is practically nil. It has been shown by numerous observers that the tissues contain a "fat-splitting" ferment, the action of which under certain circumstances may also be reversible, and it was considered probable that the blood serum contained a similar ferment. This view was first of all advanced by Hanriot [1896], who tried the action of the blood on monobutyryl. His experiments, however, are erroneous as has been pointed out by Arthus [1902] because he took no precautions to ensure aseptic conditions and also because he used monobutyryl, which can be very easily hydrolysed, and it by no means follows that ferments, breaking up such simple esters as monobutyryl, have the same effect on neutral fats. Hagemeister [1903] showed that the injection of fat between muscle fibres was followed by its presence beneath the sarcolemma. He deduced that the lipolytic ferment of the tissue fluids caused the hydrolysis of the fat, diffusion of the resulting products occurring, followed by resynthesis in the muscle.

Fischler [1902, 1903] demonstrated the deposition of "fat" in organs after the injection of soap into the blood, or the perfusion of surviving organs with blood containing soap, thus lending support to the above. Munk [quoted by Fischler, 1902, p. 149], however, has shown that soap

even in small amounts exerts a toxic effect on the medullary centres. A small amount of soap apparently does occur in the blood and according to Schultz [1897] 28 % of the total fatty acids in the blood are in the form of soaps.

There is therefore no *a priori* objection to the possibility of the blood containing a lipase acting on the fats in the above-mentioned way and of the products being rapidly diffused into the cells to be resynthesised and stored up or used as required.

Experiments, however, conducted with strict precautions as to asepsis show that the blood serum does not contain a ferment capable of splitting ordinary fats.

The experiments of Cohnstein and Michaelis [1894] showed that there was a loss of ether-soluble substances on incubating mixtures of blood and chyle and a corresponding increase in water-soluble substances, and that this was due to a ferment in the erythrocytes. Their experiments have, however, the drawbacks that they were not absolutely aseptic and that there were inaccuracies in their mode of estimation.

Their results have been in part confirmed by Hamburger [1900] who was able to demonstrate a loss of ether-soluble substance after incubating a lipanin emulsion with blood.

Weigert [1900], however, considered that Cohnstein and Michaelis' results could be explained by autolysis of the lecithin. Hepner [1898] had previously shown that the blood plasma contains cholesterol and cholesterol esters of the fatty acids, and thought that the free cholesterol might conceivably have arisen by the action of a ferment on the cholesterol ester. Weigert therefore thought that the loss in ether extractives in Cohnstein and Michaelis' experiments could be accounted for by this, since the liberated fatty acids would become converted into soaps and thus no longer be ether-soluble. He experimented with blood plasma, blood and corpuscles alone. He found that blood plasma on incubation suffered no loss; when blood was incubated a loss of 18 % of ether extract occurred; whereas when corpuscles alone were thus treated there was a loss of 40–50 % of ether extract, and since Hepner and Hoppe-Seyler [quoted by Hepner, 1898] showed that red cells contained no cholesterol ester or neutral fat, the resulting change can only have been due to hydrolysis of the lecithin.

In the experiments of the author the following methods of extracting fat were adopted:

1. Drying the material with sand over a water-bath, and extracting with ether in a Soxhlet apparatus for 48 hours.

2. Mixing with anhydrous sodium sulphate as recommended by Pinkus, finely powdering the resulting dry mass, and extracting with ether at the room temperature for several days, with vigorous shaking at intervals. The extraction was repeated with fresh ether till nothing further was extracted.

3. Mixing with 94% alcohol and keeping at 37° for several days, then filtering and repeatedly extracting the precipitate with alcohol, and finally boiling up. The united extracts were then evaporated and extracted with 94% alcohol. The original residue was also extracted with ether, and the resulting extracts were divided into their constituents: fats, fatty acids and lecithin.

4. Digesting with pepsin and hydrochloric acid; evaporating with sand and extracting in the Soxhlet with ether.

Precautions to preserve absolute asepsis were taken in all these experiments. The various gases were allowed to bubble through the mixtures after having been filtered through sterile cotton wool. All specimens showing bacterial contamination, either by direct or cultural examination, were discarded.

I. CHANGES OCCURRING WHEN BLOOD ALONE IS INCUBATED.

Exp. 1.

100 c.c. of blood (by sand and ether extraction) yielded	...	...	0.403 g. fat
100 ,, ,, incubated at 37° for 24 hours in closed vessel yielded	...	...	0.381 ,,
100 ,, ,, same incubation, but with air current	...	...	0.297 ,,
Loss in specimen in air current = 26.3 %.			
,, without ,, = 5.4 %.			

Exp. 2.

100 c.c. of blood by sand and ether extraction yielded	...	...	0.366 g. fat.
100 ,, ,, incubated in air current for 24 hours at 37°	...	...	0.295 ,,
100 ,, ,, incubated without air at 37° for 24 hours	...	...	0.299 ,,
Loss in air current = 19.4 %.			
,, without ,, = 18.3 %.			

Exp. 3. Extraction after drying with anhydrous sodium sulphate.

(a) 100 c.c. of blood contained	...	...	0.24 g. fat.
100 ,, ,, after incubation in air current for 24 hours at 37°	...	...	0.184 ,,
contained	...	...	0.184 ,,
Loss = 23.3 %.			
(b) 100 c.c. of blood contained	...	...	0.328 g. fat.
100 ,, ,, treated as in (a) contained	...	...	0.276 ,,
Loss = 15.9 %.			

For the purpose of determining to what the loss was due, the following estimations were made. The blood was extracted with alcohol and the residue with ether, the alcoholic extract was acidulated with dilute mineral

acid evaporated and shaken out with ether. The ether was freed from mineral acid by repeatedly shaking with water. The ethereal residues were united, evaporated, dried and weighed. The lecithin was determined by precipitation with acetone, the fatty acids by titration with N/10 alcoholic potash, and the neutral fat subsequently by boiling with a known quantity of N/10 absolute alcoholic potash.

250 c.c. of blood were taken.

Unincubated blood		Incubated blood
Total weight of residue	0.958 g.	0.936 g.
Lecithin	0.28	0.127
Fatty acids	0.152 (5.4 c.c. N/10 alc. pot.)	0.245 (8.7 c.c.)
Neutral fat	0.316 (11.2 „ „)	0.310 (11.0 c.c.)
Cholesterol	0.102	0.122

With the exception of the increase in the cholesterol figure the whole difference is in the lecithin and fatty acids—the loss in the lecithin is 0.153 g., and the increase in the fatty acids is 0.093 g. Now if lecithin contains about 60 % fatty acids, then 0.153 g. contains 0.092 fatty acid.

Several other experiments showed a similar change.

II. CHYLE ALONE.

Exp. 1.

50 c.c. of chyle contained (by sand and ether method)	...	0.631 g. of fat.
50 „ „ incubated at 37° without current of air	...	0.579 „
100 „ „ incubated at 37° in air current	...	1.064 „
Loss without air current = 8.2 %.		
„ in „ „ = 15.7 %.		

Exp. 2. *Extracted by the Sodium Sulphate-Ether method.*

25 c.c. of chyle contained	...	...	0.317 g. of fat.
100 „ „ incubated in air current contained	...	...	1.178 „
Loss = 7.1 %.			

Exp. 3. *Alcohol-ether extraction.*

100 c.c. of chyle	(a) Unincubated	(b) Incubated
Ethereal extract of the original extract	1.356 g.	1.243 g. (loss = 8.4 %)

After acidulating the residue of the original extract, shaking out with ether and removing mineral acid and adding the ethereal extracts together;

Lecithin found	0.056 g.	0.014 g.
Fatty acids	0.0	0.034
Neutral fat	1.208	1.137

From this it would appear that there was some hydrolysis of the lecithin and loss of a small amount of neutral fat. The fatty acid increase can be explained by the hydrolysis of the lecithin, since 0.048 g. lecithin contains about 0.029 g. of fatty acid.

III. EXPERIMENTS ON THE INCUBATION OF A MIXTURE OF CHYLE AND BLOOD.

(1) The sand method of extraction.

Exp. 1.

100 c.c. of a mixture of equal parts of chyle and blood were found to contain	1.281 g. of fat.
100 c.c. of same mixture incubated for 24 hours without air current were found to contain	1.200 "
100 c.c. of same, incubated in an air current for 24 hours contained	0.9155 "
100 c.c. of same, incubated in an air current for 48 hours contained	0.8025 "
Loss was therefore:—in incubation without air current	6.4 %.
in 24 hours incubation with air current	28.5 %.
in 48 " " " "	37.3 %.

Exp. 2. Performed as above, showed;

100 c.c. of a blood chyle mixture contained	1.41 g. of fat.
100 c.c. of same incubated in air current at 37° for 24 hours	0.536 "
100 c.c. of same, incubated for 48 hours, same way	0.373 "
Loss after 24 hours was	61.9 %.
" 48 " "	73.5 %.

(2) Sodium Sulphate Method.

100 c.c. of a mixture of blood and chyle contained	1.228 g. of fat.
100 c.c. of the same mixture, incubated in a current of air at 48° for 24 hours	0.661 "
Loss =	46.2 %.

These figures show that on incubating chyle and blood together there is a large loss of ether extractive. This is not due to the method of extraction, it is as apparent with the one method as with the other. The loss is greater after air has been passed through the mixture, but contrary to the observation of Cohnstein and Michaelis [1894] it also occurs in the absence of an air current. The loss again is not the sum of the losses that would be obtained by incubating the blood and chyle separately.

Thus in the following experiments:

100 c.c. of blood yielded	0.29 g. of fat.
100 c.c. incubated for 24 hours with current of air at 37° yielded	0.232 "
Loss =	0.058 g. or 20 %.
100 c.c. of chyle yielded	1.202 "
100 c.c. incubated as above	1.034 "
Loss =	0.168 g. = 14.0 %.
Now a mixture of 50 c.c. of each contained	0.770 g. of fat.
But incubation as before yielded	0.478 "
Loss =	0.298 g. = 38.4 %.

Now the sum of the individual losses for 50 c.c. of each = 0.117 g., which is less than half the loss which occurred, when the two were incubated together.

This is also shown by the following experiment:

100 c.c. of chyle were found to contain	...	...	...	...	1.268 g. of fat.
100 „ „ yielded after incubation	...	...	...	...	1.168 „
Loss = 0.1 g.					
100 c.c. of blood yielded	...	...	...	...	0.314 „
100 „ „ after incubation yielded	...	...	...	...	0.244 „
Loss = 0.070 g.					
A mixture of 50 c.c. of chyle and 25 c.c. of blood were estimated to contain	...	...	...	...	0.712 „
After incubation a similar amount yielded	...	...	...	...	0.329 „
Loss = 0.38 g. = 53.8 %,					

whereas the sum of the losses for each separate incubation

$$= 0.05 + 0.0175 = 0.0675 \text{ g.}$$

The loss is thus considerably greater than the sum of the individual losses.

(3) To see if the serum contained the substance which caused this loss of substance extractable by ether a mixture was made of 100 c.c. of chyle and 100 c.c. of sheep's serum.

100 c.c. of the mixture contained	...	...	...	...	0.663 g. of fat.
100 „ „ „ after incubation in an air stream after 24 hours yielded	...	...	...	...	0.591 „
Loss = 0.072 g. = 10.8 %.					

Similar experiments give like results up to 15 %.

(4) In order to determine whether the change is due to a ferment in the red corpuscles or other formed constituents, the following experiments were performed with carefully washed erythrocytes. These were obtained by centrifuging whipped blood and washing six times with sterile normal saline in sterile centrifuge tubes.

Exp. 1.

50 c.c. of chyle and 5 c.c. of red cells contained	...	...	...	...	0.802 g. of fat.
100 „ „ 10 „ „ incubated for 24 hours in an air current	...	...	...	...	...
yielded	...	...	...	...	1.172 „
Loss = 0.43 g. = 26.9 %.					

In the next part of the experiment the chyle was first heated to 80° for 15 minutes:

100 c.c. of this with 10 c.c. of the suspension of red cells incubated for 24 hours	...	...	...	...	...
at 37° yielded	...	...	...	...	1.107 g. of fat.
Loss for unboiled chyle was 26.9 %.					
„ „ boiled	...	...	...	...	30.9 %.

Exp. 2.

100 c.c. of chyle yielded	...	...	...	...	1.825 g. of fat.
20 c.c. of red corpuscles yielded	...	...	...	...	0.048 "
100 c.c. of chyle + 20 c.c. of the corpuscles incubated for 24 hours in an air current yielded	...	...	...	...	1.284 "

Loss was 31.4 %.

Exp. 3.

50 c.c. of chyle yielded	...	...	...	...	0.912 g. of fat.
20 c.c. of corpuscle suspension yielded	...	...	...	...	0.048 "

Both were heated to 80° and then incubated at 37° for 24 hours. The total amount of ether extract found was 0.937 g. The loss was therefore practically nil.

It will thus be seen that probably the red cells, or substances centrifugalised down with them, have the power of causing this loss of ether extractive.

(5) The next question investigated was whether this power was due to the haemoglobin, to a ferment in the cells, which may be to some extent diffusible into the plasma, or as stated above to elements precipitated by the centrifuge with the red corpuscles.

This was investigated by obtaining crystals of haemoglobin from dog's blood. The blood was whipped and centrifugalised, the corpuscles were then repeatedly washed with normal saline and then laked with ether, centrifugalised and washed with dilute alcohol and ammonium sulphate solution.

Exp. 1.

50 c.c. of chyle + 25 c.c. of a watery Hb solution yielded	...	...	...	0.550 ether ext.
Twice this amount was incubated at 48° for 24 hours in air current and yielded	...	...	...	0.729 "

Loss was 0.371 g.

Exp. 2. The haemoglobin was previously treated with a saturated solution of sodium fluoride in a saturated ammonium sulphate solution. The crystals were then repeatedly washed with a saturated solution of ammonium sulphate solution to remove the sodium fluoride.

50 c.c. chyle together with 50 c.c. watery Hb solution yielded	...	...	0.665 g. of fat.
The same amount after 24 hours incubation in a stream of air at 48° yielded...	...	...	0.632 "

Loss was 0.033 g. = 5 %.

Exp. 3.

100 c.c. of chyle was mixed with 50 c.c. of a watery solution of Hb. The Hb had been previously dried for several days at 50°. The mixture contained	...	...	1.268 g. of fat.
The same amount was incubated for 24 hours and yielded	...	...	1.202 "

Loss = 0.056 g. = 5.2 %.

To elucidate this point, whether the loss was due to the action of the haemoglobin, a mixture of chyle and blood was incubated and a mixed stream of carbonic oxide and oxygen gases was passed through the fluid. The carbonic oxide converted the haemoglobin into carboxy-haemoglobin and the oxygen was available if necessary.

By previous experimentation it was determined that the "lipase" obtained from pig's liver was unaffected by prolonged exposure to a stream of carbonic oxide gas as regards its action on ethyl butyrate.

The blood used was first saturated with carbonic oxide.

Exp. 1.

50 c.c. of blood and chyle yielded	...	...	0.490 g. of ether ext.
50 c.c. of the same mixture after incubation yielded	...	...	0.390 ,, ,,
Loss was 0.1 g. = 20.4 %.			

Exp. 2.

100 c.c. of a mixture of equal parts of blood and chyle yielded	...	...	1.096 g. of ether ext.
The same amount after similar treatment yielded	...	...	0.731 ,, ,,
Loss was 0.365 g. = 33.3 %.			

The experiments show that the change can still occur when the haemoglobin is put out of action as an oxygen carrier.

In these experiments it was certain that the haemoglobin was converted into carboxy-haemoglobin because of the colour of the mixture.

In the method of preparation of the haemoglobin crystals, proteins in the corpuscles or substances in the plasma like blood, dust particles, and leucocyte debris may be precipitated so that any possible ferment in connection with them would be precipitated as well. The action of the carbonic oxide and sodium fluoride leads to the supposition that there is a ferment separate from the haemoglobin.

(6) The following experiments were performed to see if the oxygen is really a necessity, or if the air current acts simply mechanically, thoroughly mixing the blood and chyle. The mixtures were incubated for 24 hours at 37°.

(a) 50 c.c. of a mixture of blood and chyle yielded	...	...	0.350 g. of fat.
100 c.c. of this mixture after treatment in coal gas yielded	...	...	0.542 ,,
Loss was therefore 0.158 g. = 22.6 %.			
(b) 50 c.c. of blood and chyle contained	...	...	0.450 ,,
100 c.c. incubated in a stream of hydrogen gas contained	...	...	0.696 ,,
Loss was 0.204 g. = 22.5 %.			
(c) 50 c.c. of blood and chyle mixture contained	...	...	0.395 ,,
100 c.c. of the same mixture in a hydrogen stream yielded	...	...	0.608 ,,
Loss was 0.182 g. = 23 %.			
(d) 50 c.c. of chyle + 25 c.c. of blood contained	...	...	0.673 ,,
The same mixture incubated in air stream yielded	...	...	0.229 ,,
The same mixture incubated in CO ₂ stream yielded	...	...	0.478 ,,
Loss in air = 0.444 = 66 %.			
,, CO ₂ = 0.195 = 29 %.			

Thus it would appear that oxygen aids the action of the ferment, but on comparing with the result in (c) of this series it is obvious that mechanical agitation by the passage of a current of any gas also appreciably aids the process.

(7) The influence of protoplasmic and ferment poisons was next examined.

Exp. 1.

- (a) 100 c.c. of a mixture of chyle and blood yielded 0.708 g. of fat.
 100 c.c. of same mixture containing 5 % sodium fluoride was incubated
 for 24 hours in air stream at 48° and then contained 0.657 ,,
 Loss was 0.051 g. = 7 %.
- (b) 100 c.c. of the same mixture with potassium cyanide added and incubated
 in the same way yielded 0.641 ,,
 Loss was 0.067 = 10 %.
- (c) 200 c.c. of the same mixture was incubated in the ordinary way and yielded 0.547 ,,
 Loss was 0.869 = 62 %.

(8) Some experiments were performed to determine the fate of the individual constituents.

Exp. 1. A mixture of equal parts of blood and chyle was made. (a) 100 c.c. were directly dried with sand and extracted with ether in the Soxhlet; (b) 100 c.c. were similarly treated after incubation in an air current at 37° for 24 hours.

(a) Unincubated		(b) Incubated	
Total residue	0.732 g.		0.416 g.
Neutral fats	0.583 (19.8 c.c.)	0.327	(11.1 c.c. N/10 alk.)
Fatty acid	0.0		0.0
Lecithin	0.096		0.032
Cholesterol	0.053		0.057

There is thus, as was expected, a loss in neutral fat. The dried residue was then extracted with alcohol in the incubator at 37° while being continuously shaken, the alcoholic extract was evaporated and treated with mineral acid, and shaken out with ether, the ether subsequently being washed with water to remove the mineral acid. The ethereal extract was then titrated with N/10 alkali in alcohol.

The unincubated required 0.8 c.c. = 0.0226 g. fatty acid.

The incubated required 3.1 c.c. = 0.0874 g. fatty acid.

On subsequently boiling the extract of the incubated specimen with a measured amount of N/10 alcoholic potash, it was found that 5.8 c.c. were used up = 0.1709 g. of fat, which had been liberated. This fat scarcely contained any trace of phosphorus. Hence the alcohol liberated a certain amount of neutral fat, which had become ether-insoluble to direct ethereal extraction.

Other experiments gave similar results as regards the amount of fatty acids present in the ether extract of the second alcoholic extraction, and showed that neutral fat was also present which did not come out in the original extraction.

Exp. 2. *A mixture of chyle and blood treated as in Exp. 1.*

	Non-incubated	Incubated
Total residue	0.746 g.	0.402 g.
Neutral fat	0.5938 (20.2 c.c. N/10 alk.)	0.294 (10 c.c.)
Fatty acid	0.0	0.0
Lecithin	0.99	0.029
Cholesterin	0.068	0.073
<i>Subsequent extraction as above.</i>		
Fatty acids	0.0328 g. (1.2 c.c.)	0.093 g. (3.3 c.c. alk.)
Fats	0.0	0.138 (4.7 „)

The same experiment was made with the same materials, but instead of subsequent alcoholic extraction the finely ground material after the ether extraction was digested with pepsin and hydrochloric acid and then dried and extracted with ether.

Exp. 3. Thus 100 c.c. of a mixture of blood and chyle were examined.

	(a) Unincubated	(b) Incubated
Primary extract	0.753 g.	0.449 g. (loss = 43.1 %)
Secondary „	0.028	0.32
Fatty acid in secondary extract	trace	0.0789
Neutral fat	nil	0.233 (7.9 N/10 alkali)

The same method as above was used. 200 c.c. of a mixture of blood and chyle were used for immediate estimation by ether extraction followed by treatment with pepsin and extraction, and two separate lots of 200 c.c. were incubated in an air current at 37° for 24 hours, one examined by ether-alcohol extraction, and divided into its constituents, the other first extracted with ether and then digested with pepsin and acid and again extracted with ether.

Exp. 4.

	Control	Specimen "A" ether-alcohol extraction	Specimen "B" digestion with pepsin, etc.
	g.	g.	g.
Total residue (first extract)	1.64	0.813 (loss = 50.4 %)	0.799 (51.8 %)
Neutral fats ...	1.3934 (47.2 c.c. N/10 alk.)	0.705 (23.9 c.c. N/10 alk.)	—
Fatty acids ...	0.0	0.0	—
Lecithin ...	0.198	0.087	—
Cholesterol etc. ...	0.0486	0.021	—
Fatty acid in second extract	0.0479 (1.7 c.c. N/10 alk.)	0.121 (4.3 c.c. N/10 alk.)	0.1325 (4.7 c.c.)
Fat in second extract ...	nil	0.545 (18.5 c.c.)	0.7139 (24.2 c.c.)

It was also found that by extracting with alcohol on the water-bath and then with ether, the whole of the ether-insoluble fat could be recovered.

Two lots of 100 c.c. of a mixture of chyle and blood were examined (a) unincubated, (b) incubated in the usual way.

	(a) Unincubated	(b) Incubated
Primary extract	0.712 g.	0.467 g.
Secondary „	0.028	0.298

These experiments further show that the neutral fats are not hydrolysed by the blood or chyle, but that the lecithin is the only fat hydrolysed by the ferments in the chyle or blood. This is at variance with the observation of Hamill [1907] who states that the chyle can hydrolyse a neutral fat, viz., olive oil.

(9) The question then arose if this ether-insoluble fat could be isolated and its constitution determined and especially if it were diffusible and soluble in water.

The method adopted was that of Cohnstein and Michaelis [1894], viz. diffusion through parchment thimbles into distilled water. In all the experiments it was found that only soaps, etc., diffused through, and no substance containing the neutral fat, and that the amount of fatty acid thus obtained corresponded to the degree of hydrolysis of the lecithins.

Experiments were also made with a lipanin emulsion, made with sterile ascitic fluid and lipanin. A very fine emulsion was produced.

It was found that on incubation with blood and a current of air the same results were obtained as above with chyle fat and that the fat could be recovered again by treatment with alcohol or pepsin and hydrochloric acid.

These observations are in accordance with those of Mansfeld [1907] who showed that the fat in lipanin after incubation with blood became non-extractable by ether, but could be recovered by Liebermann's method.

Hamburger [1900] was only able to obtain this result when he made his artificial chyle with a chyloid fluid. In these experiments, as in those of Mansfeld, ordinary ascitic fluid worked well.

CONCLUSIONS.

It would seem justifiable to draw the following conclusions:

1. Blood and chyle contain a ferment which can hydrolyse lecithin but not neutral fat.
2. When blood and chyle fat are incubated together, the neutral fat forms an absorption combination with the proteins and is thus rendered non-extractable by ether. The combination can, however, be broken up by peptic digestion, or treatment with alcohol. It is not formed during the process of drying.
3. The combination appears to be broken up by heat, since larger losses are obtained if the incubated mixtures are not heated, and if the material be dried at a low temperature.

4. The formation of this complex appears to be due to the formed elements of the blood, since the corpuscles or material carried down with the corpuscles on centrifugalisation have the same effect, and serum by itself has not.

5. The action does not appear to be due to the haemoglobin, but to some material precipitated in the method of preparing the haemoglobin, since on rendering the haemoglobin inert as an oxygen carrier by carbon monoxide, the change still occurs.

6. The change occurs best in a current of air or oxygen, and if the mixture be thoroughly agitated.

7. The combination appears to be due to the action of a ferment because:

- (a) the haemoglobin may be rendered inert without affecting the process;
- (b) ferment poisons seriously interfere with it.

8. Similar combinations may occur when blood is mixed with a very fine emulsion of fat. The compound, however, does not appear so stable since it is practically always broken up by heating and by alcohol.

9. The albumin-fat combination is not diffusible nor soluble in water. After incubation an increase in diffusible material takes place, but this is only due to the diffusion of the products of the lecithin hydrolysis, viz., glycerophosphoric acids, soaps, choline.

10. The chyle and corpuscles contain a ferment capable of breaking up lecithin and liberating the fatty acids.

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