A STUDY OF THE PROCESSES OF FAT METABOLISM AND METHODS FOR DETERMINATION OF FAT

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Historical*

Part I

Our present conception of how fat is absorbed is indeed very modern. The most work has been done within the last fifteen or twenty years. Before going into the detailed account of the work of more of the modern investigators, however, I shall take the liberty to present in a condensed form the theories and conceptions of digestion and absorption from the ancient times to the present. I hope that this will present a broader view of the subject and help us understand the more modern tendencies.

In ancient Egypt it was believed that all diseases arose from food. The Hindus recognized that digestion is going on in the stomach and small intestine and describe a demon "Pachaka" the fire of digestion, situated between the stomach and small intestine. The Chinese regarded (and the majority probably still does) the bile as the seat of courage and the stomach as the abode of the mind. The ancient Hebrew regarded health and disease as emanating from the same divine source.

*References to all authors cited in this paper will be found at the end, arranged alphabetically.

The pancreas was considered an accessory organ of the liver.

The stomach performed a purely mechanical function, that of churning the food; it is compared to a mill. They recognized, however, that the main digestion is carried on in the intestine.

Plato (427-347 B.C.E.) greatly propounded the theory that some of the fluid drunk passed thru the wind pipe into the lungs and there served to cool the heart.

Aristotle ( ) said that 'heat is innate in the heart placed there by the Creator'.

The dark ages, naturally, also had their effect on medicine. People followed blindly. Nothing was accomplished in respect to the physiology of digestion and absorption. Here and there we find some one expresses a truth, but it becomes lost in the great chaos.

John Baptismo Van Helmont (1577-1644) seems to be the first to look upon digestion as a process of fermentation.

About the beginning of the eighteenth century Dr. Pitcairn trying to solve the problem of digestion, estimated the pressure of the walls of the stomach as 12,951 pounds, or over 6 tons, and ascribed gastric digestion to this triturating force alone. In his essay he states that this explanation is quite sufficient and that there is no need to call in the assistance of a demon or stygian liquor (I suppose he must have referred to the gastric juice).

We do not find much writing about digestion and absorption till we come to the beginning of the nineteenth century.

Dr. Brousais (1828) speaking about gastric juices, states : "The fact if established, of there being in certain animals a superadded glandular apparatus for the secretion of what is called gastric juice, would in our minds be a strong argument against the admission of such a juice in animals in which like man there are no such glands to be seen. When we bear in mind the circumstance of the liver surrounding the stomach of some of the smaller and inferior animals and emptying its secretion into the cavity by several ducts, we may be allowed to be skeptical on the subject of gastric juice, which properties must have them materially affected, if not entirely altered, by the admixture of the bile." This same author in another place states that the precise mechanism by which absorption is performed is as yet an unsettled question. He attributes absorption mainly to the passage of fluids from less dense to more dense solutions.

Robley Dungleson (1836) quotes Montegre', who in 1812 after a series of experiments which he presented to the French Academy, came to the conclusion that there was no such a thing as gastric juice, and what was up to then considered gastric juice was nothing more than common ordinary saliva.

Dungleson, as well as most of the authors of his time, believed that the gastric secretion acted an all substances alike. There were different theories proposed as to how the stomach can decompose all substances. Some compared it to a fermenting vat, others thought it acts like a stew pan, etc., etc.

Dr. Wm. Hunter (quoter by Dungleson) who may have been either dissatisfied with the various theories, or who probably could not help admiring the mysterious work of the stomach, once said, "Some physiologists will have it, that the stomach is a mill; others that it is fermenting vat; others again that it is a stew pan; but in my view of the matter, it is neither a mill, a fermenting vat nor a stew pan; but a stomach, gentlemen, a stomach."

At this period the different authors seemed to have had the true conception that the bile and pancreatic juice had some influence on fat absorption, especially the former. Most of them, while considering the bile as important, did not deem it an essential agent in digestion. Dr. Franz Simon (1840) says: "Let us hope that we may some day be able to explain the mysterious function of the bile." He quotes Brodie (1823) and Tiedeman and Gmelin, that the bile has no influence on digestion or chylification. The bile was considered by them as an excretion necessary to the body like carbon dioxide. urin, uric acid, etc. Speaking about the pancreatic juice he says, "We are unable to say how the pancreatic juice which differs so greatly chemically from saliva, takes part in the change which goes on in the food in the intestinal canal."

Carlo Matteucci (1848) has the following to say: "We have seen that the bile performs little or no part in digestion." Liebig (who dominated the field of Chemistry from 1840 to 1860) assumes that the bile poured into the duodenum forms a soluble combination with potash, which is ab-

sorbed and converted into carbonate of potash by yielding up a portion of its carbon to the oxygen.

Claude Bernard (1846) made the important discovery that the pancreatic juice was able to split fat into fatty acids and glycerol.

Lambert (1852) states, that the quantity of pancreatic juice formed, the diseases of the organ, or its importance are not known. It should be borne in mind that this statement was made after Claude Bernard found that the purpose of the pancreatic juice was to split neutral fats into fatty acids and glycerol. This same author, speaking about the bile, says: "Some believe that the gall is formed in the liver as bile. It would seem as if the gall bladder could as easily form the entire gall as add bitterness to it."

From 1850 we find the physiologists have better ideas as to the functions of the different juices, but those ideas were by no means very concise and exact. John Wm. Draping (1856) gives the purpose of the pancreatic juice as helping to emulsify the fats, and thinks that the latter are absorbed as such, a theory held until very recently, and probably still held by a few of the older physiologists. The same author speaking about absorption says: "Whatever may be the special manner by which the fats pass from the intestine into the lacteals, they have scarcely gained those vessels before they undergo a change. The quantity of free fats diminishes, and that of saponified fat increases; this is probably accomplished by soda obtained from the blood."

A very interesting statement is found in the book of Dr. Edward Jarvis (1868): "This digestive process, which effects so great a change, is wonderful, as well as interesting. This change is a vital one; at least it is effected by the fluids which are within the living organs. By what unseen agency these fluids obtain this power is known only to the all-wise Creator. It is not revealed to us nor need it be."

Foster (1878) states: "Fats undergo no change by the action of the gastric juice." "Bile has a slight solvent action on fats. It has a slight, but only slight, emulsifying power. The wetting of membranes with bile or with a solution of bile salts assists in the passage of fats through the membranes."

"Pancreatic juice has a twofold action on fats: it emulsifies them, and it splits them up into their respective acid and glycerine."

"In all probability saponification in the intestine is a subsidiary and unimportant process. In cases where all the pancreatic ducts have been ligatured, and there has been no reason to think that pancreatic juice to any amount has entered the intestine, fat may be present in the lacteals, consequently the digestion of the fat must be referred to the bile."

It is to be noted that Foster was an accurate observer, and many of his conclusions are more or less true.

Victor Vaughan (1880) has the same to say about fat digestion and absorption as Foster.

It is to be seen that our modern theories had not been conceived until about 1880. It shall be my purpose from now on to go into a more detailed account of the work of modern investigators, compare their results, and summarize the different theories regarding fat metabolism.

Part II

Organs.

Before going into a detailed account of what organs of the body play a part in fat absorption, and how they play that part, it is necessary to state the several theories regarding fat absorption. The theory in vogue for a long time was, that fat, no matter in what form it was fed, could be absorbed as such (Huxley, 1878), and the main proof given in its support was that on making an histological examination of the epithelial cells fat could be plainly seen. However, this theory is now almost obsolete, and I shall not say much more about it. The two theories at present are, first, that fat in emulsified form can be absorbed (Hoffbauer 1900). This theory has many supporters, even at the present time. The other theory proposed is that fats must be split first into their fatty acids and glycerol before they can be absorbed.

I should mention here that another theory of fat absorption was proposed by Connstein and Michaels (1898) and that is, that the fat changes from the impermeable form to a permeable one, as is the case with the albumins, which become dyalizable thru peptonization and as those peptonized forms immediately after passing thru the epithelium are converted back into albumins, so also the permeable fats are changed back to neutral fats. This theory, as far as I know, has no supporters, and most of the modern investigators do not mention it.

Stomach

The first organ for consideration is naturally the stomach. This organ has not until very recently been recognized

as having any influence on fat metabolism. It is therefore not at all astonishing to find most of the text books stating that the stomach plays no part at all in the digestion and absorption of fat; but the text books are not the only ones in this respect. Dr. Noel Paton (1911) says "There is no evidence that fats are absorbed under any conditions in the stomach." However, if one only examines the literature on the subject he must come to the conclusion that Paton some way or other overlooked the latest developments on the subject.

S. Levites (1906) states that in the stomach fat undergoes only little chemical change so long as the liquid from the duodenum does not pass into the stomach. "When this takes place, splitting is considerable. Fat is not absorbed in the stomach." However, he himself states that egg yolk in the stomach is saponified in rather large amounts, for which he can give no reason.

E. S. London (1906-7) and several other investigators, notably Contejan, Kunkel, Aldor (quoted by London) do not accept the view that there exists a special gastric enzyme, and that splitting in the stomach is due either to bacterial action or to antiperistaltic movements of the pancreatic juice from the duodenum. London pointed out that with gastric juice, which he obtained by means of a pyloric fistula, mixed with the yolk of an egg, he was only able to obtain a splitting between 2-5 per cent, while the splitting of the same material in the stomach may amount to as high as 23 per cent. So he concludes that the splitting in the stomach is arrested by the antiperistaltic in-

flow from the duodenum.

London and Wersilowa (1908) who experimented on dogs and obtained the juice by means of fistulas, concluded that fat splitting occurs in the stomach to the extent of 32 per cent in 6 hours. They admit a stomach lipase, but state that the action is very little, the most splitting being accomplished by the regurgitated duodenal juice, and for this very reason stomach lipase is still an undecided question.

Klemperer and Scheurlen (1889) also claimed that fat is not attacked in the stomach. (It should be mentioned here that they worked with non-emulsified fat.)

Boldyreff (1904) states that a fat rich food is a cause for regurgitation of pancreatic juice into the stomach.

Connur and Niclaux (1930) claim that digestion of cotton seed oil in the stomach is of little importance, being perhaps 5 per cent in a time varying from 1 to 2 hours. "The gastric digestion of fat has been supposed to be due to a lipase or to a reflux of duodenal digestive juices into the stomach. Refluxes in the stomach caused a digestion of from 7.2 to 16.8 per cent." "If there is a lipase in the stomach to help digestion its action is very little indeed." "Duodenal juices flowing back into the stomach digest fat there and, further, fats already in the process of digestion can flow back as the free fatty acids." They also claim that in a mixture of fats and duodenal juices fermentive action can proceed after reflux into the stomach.

Usuki (1910) claims that cleavage of lecithine in the stomach occurs more quickly than that of neutral fats.

Volhard (1900) claims that he was able to show a true stomach lipase which is derived from the fundic region of the mucous membrane of the stomach. To substantiate his claims that he was dealing with an enzyme, he filtered the extract of the fundic region thru a clay filter, and was able to find the enzyme in the filtrate. Volhard's contention has been substantiated by many of the modern investigators, notably Weiss (1912), Fromme (1903), Fallaise (1907), and Zinsser (1906).

The only contention at present is, to what extent can splitting go on in the stomach?

Many have proved that absorption takes place to a large extent in the stomach. Among these investigators are Greene (1912-13), Weiss (1912), and Hartley (1909).

Binech and Gugot (1904) showed that human gastric juice in the presence of weak hydrochloric acid solution was able to split monobutyrin. The presence of alkali or strong acids was injurious. Glycerol extracts of the cardiac region act analogous to the fresh juices. According to Kastle and Loevenhart, splitting of neutral non-emulsified fat by the gastric juice is doubtful. Connstein concludes that there is a splitting ferment in the stomach.

Von Pesthy (1911) would not ascribe cleavage of fat in the stomach to antiperistaltic movements from the duodenum, for the enzyme would become inactivated by the free hydrochloric acid.

Zinsser (1906) experimented on man. He gave them for break-

fast a suspension of egg yellow in bouillon. After a time he withdrew a certain amount of fluid from the stomach, shook it up with ether, added alcohol, and titrated it with tenth-normal sodium hydroxide, using phenolphthalein as indicator. The number of cubic centimeters of alkali used he calls value I. He then added 10 c.c. of normal sodium hydroxide, let it stand for 24 hours. (He assumed that the neutral fat is all saponified during that time). He next added 10 c.c. of normal sulfuric acid, which besides neutralizing the excess of sodium hydroxide sets free the fatty acids. These are again titrated. He calls the number of cubic centimeters used the second time value II. He calculated the percentage of splitting from the following formula:

$$X = \frac{\text{Value I}}{\text{Value I} + \text{Value II}} \times 100$$

It should be noted that the method will not give strictly reliable results. The lower acids like acetic and propionic are soluble in ether, and it seems hardly fair to titrate them along with higher fatty acids. However, the results in general show fairly well that Zinsser dealt with fat splitting enzymes.

Table I

	Date	Name	Food fed	Length of Digestion	Remainder in Stomach	Amt. of Ether added c.c.	Amt. of alcohol added c.c.	Amt. of Ether used c.c.	1st. Titration c.c.	2nd Titration c.c.	Sum of both Titrations c.c.	Fat Split by Enzymes %
I	20/8	Moses R. 25 yrs. (Neurasthenia)	Test breakfast	1 hr.	100 c.c. of a well digested fluid							
A	22/8	"	300 c.c. of bouillon and 2 egg yolks	2 hr.	10 c.c. of fluid with a fine precipitate washed out With 100 c.c. of water	100	5	60 colorless	1.85	6.0	7.85	23.6
B	23/8	"	"	2 hr.	15 c.c. fluid with a fine precipitate washed with 100 c.c. of water	50	2	35	2.4	15.7	18.1	13.2

Table II

	Date	Name	Food fed	Length of Digestion	Remainder in Stomach	Amt. of Ether added c.c.	Amt. of Alcohol added c.c.	Amt. of Ether used c.c.	1st Titration c.c.	2nd Titration c.c.	Sum of both Titrations c.c.	Fat Split by Enzymes %
II	20/8	Karl B. 25 yrs.	Test breakfast	1 hr.	120 c.c. well digested							
A	21/8	"	300 c.c. bouillon and 2 egg yolks	2 hr.	25 c.c. fluid fine precipitate	50	2	35 light yellow	1.6	3.5	5.1	31.4
B.	23/8	"	"	1½ hr.	60 c.c. fluid with fine precipitate	50	2	35 light yellow	1.8	6.5	8.3	21.6
C	29/8	"	"	2 hr.	40 c.c. with fine precipitate	50	2	40	1.0	2.6	3.6	28.3

Table III

	Date	Name	Food fed	Length of Digestion	Remainder in Stomach	Amt. of Ether added c.c.	Amt. of Alcohol added c.c.	Amt. of Ether used c.c.	1st Titration c.c.	2nd Titration c.c.	Sum of both Titrations c.c.	Fat Split by Enzymes %
II		Louise Br. (Hysteria)	Test breakfast	1 hr.	110 c.c. well digested fluid							
A	19/8	"	250 c.c. bouillon and 2 eggs on bread	1 hr.	260 c.c. well digested fluid and a few starch crumbs	75	2	40 light yellow	4.4	11.4	15.5	25.2
B.	22/8	"	"	1½ hr.	240 c.c. well digested fluid and a few starch crumbs	75	2	50 light yellow	1.5	4.3	5.8	25.8

Table IV

	Date	Name	Food fed	Length of Digestion	Remainder in Stomach	Amt. of Ether added c.c.	Amt. of Alcohol added c.c	Amt. of Ether used c.c.	1st Titration c.c.	2nd Titration c.c.	Sum of both Titrations c.c.	Fat Split by Enzymes %
IV	20/8	Marie R. 52 yrs. (Ptose-ulonia)	Test break-fast	1 hr.	140 c.c. fairly well digested							
A	21/8	"	300 c.c. bouillon and 2 egg yolks	2 hr.	50 c.c. with fine precip-itate	50	2	35 light yellow	1.8	3.2	5.0	36.0
B.	23/8	"	"	2 hr.	100 c.c. with fine precipitate	50	2	35 light yellow	1.2	3.9	5.1	23.5
	20 c.c. of original breakfast					50	2		0.7	17.6	18.3	3.8

The 3.8% split represents the average of control experiments of tables I, II, III, IV.

Table V

	Date	Name	Food fed	Length of Digestion	Remainder in Stomach	Amt. of Ether added c.c.	Amt. of Alcohol added c.c.	Amt. of Ether used c.c.	1st Titration c.c.	2nd Titration c.c.	Sum of both Titrations c.c.	Fat split by Enzymes %
IV C	19/9	Louise Br. (Traumatische hysteria)	5 egg yolks in 500 c.c. of a vinegar solution	1 hr.	210 c.c. emulsion not disturbed	75	2	50 golden yellow	3.6	9.7	13.3	27.1
D	20/9	"	"	1½ hr.	300 c.c. emulsion beginning to be disturbed	75	2	50 light yellow	3.4	9.9	13.3	25.6
E	21/9	"	"	2 hr.	200 c.c. emulsion beginning to be disturbed	75	2	50 light green	2.0	7.9	9.9	20.2

The tables I, II, III, IV, and V, show an average of splitting of about 25 per cent. Zinsser also gives data to show that in a very acid stomach the fat splitting is less, while in an achyle stomach the splitting may reach as high as 45 per cent. He concludes that the splitting is produced by a definite enzyme in the mucosa of the stomach wall.

Fromme (1903), working with glycerol extracts of the different parts of the stomach wall, was unable to show gastric lipase in the pyloric end of the stomach, and he brings this as an argument against those who believe that splitting is due to a pancreatic enzyme, since the regurgitated pancreatic juice strikes the pyloric end of the stomach first. He also puts this forward as an argument against the theory that the splitting in the stomach is due to bacterial action, since the same action ought also to go on in the pyloric end.

The method pursued by him was to mix three egg yolks with 100 c.c. of water , adding to this a glycerol extract of stomach (control experiments were conducted), and digesting a number of hours on the water bath. He next shook it with ether and 2 c.c. of alcohol and proceeded as in the method of Zinsser (page 13).

Table VI

Experiments with Glycerol Extracts from the Mucous Membrane of three Hogs.

	No. Days extracted	Length of Digestion hrs.	1st Titration of Fat split by Enzyme	2nd Titration of Fat split by saponification	Sum of Fatty Acids in 50 c.c. Ether	% of Fats split by Enzymes
Fundic Glycerol Extract No. 1						
25 c.c. egg yolk + 10 c.c. extract	2	6	1.3	Not saponified		
33 c.c. " " + 10 c.c. "	5	6	7.75	53.65	60.4	12.8
20 c.c. " " + 10 c.c. "	7	6	20.80	41.2	62.0	33.0
20 c.c. " " + 10 c.c. " boiled	7	6	1.6	44.0	45.6	3.4
20 c.c. " " + 10 c.c. " filtered	17	13	1.6	46.7	48.3	3.4
Control Experiments						
10 c.c. of fundus glycerol + 20 c.c. H_2O	--	6	0.5	2.9	---	---
10 c.c. " " " + 20 c.c. "	--	6	0.6			
Putting on more Glycerol after 7 days' extraction						
20 c.c. Egg yolk + 10 c.c. extract	3	5	9.0	37.2	46.2	19.4
20 c.c. " " + 10 c.c. "	6	7½	13.7	34.8	48.5	28.5
Pouring on more Glycerol after 13 days' extraction						
20 c.c. " " + 10 c.c. "	4	14	1.9	30.0	31.9	5.9
20 c.c. " " + 10 c.c. "	5	15	3.2	46.5	49.7	6.4
20 c.c. " " + 10 c.c. "	8	16	4.7	61.8	66.5	7.1
20 c.c. " " + 10 c.c. "	21	24	5.4	42.7	47.1	11.4
Pylorus Glycerol Extract						
33 c.c. " " + 10 c.c. "	5	6	2.8	57.0	59.8	4.6
20 c.c. " " + 10 c.c. "	7	6	2.8	59.4	62.2	4.5
20 c.c. " " + 10 c.c. "	17	14	3.3	54.0	57.3	5.7

Table VI shows that the glycerol extract of the fundic region in 6 hours is capable of splitting the fat to the extent of 33 per cent, while the same extract boiled has practically no effect. The table also shows very strikingly that the pyloric region has relatively no enzymatic action.

Table VII

Experiments with Autolyzed Mucous Membrane, obtained from the stomachs of three pigs (same as in Table VI)

	No. Days extracted	Length of Digestion hrs.	1st Titration of Fat split by Enzyme	2nd Titration Fatty Acids Split by saponification	Sum of Fatty Acids in 50 c.c. Ether	% of Fats split by Enzymes
A		Autolyzed Fundus				
20 c.c. egg yolk + 10 c.c. water extract	1	4	2.9	44.8	47.7	6.1
25 c.c. " " + 10 c.c. " "	4	6	3.9	No saponification		
30 c.c. " " + 10 c.c. " "	6	6	2.5	"	"	
30 c.c. " " + 10 c.c. " " + membrane	8	6	2.8	42.6	45.4	6.0
20 c.c. egg yolk + 10 c.c. " " + membrane	15	5	3.0	45.6	50.3	6.1
20 c.c. egg yolk + 10 c.c. glycerol extract of membrane	15	5	3.2	47.1		6.3
20 c.c. egg yolk + 10 c.c. " " of autolyzed fundus (28 hrs. in incubator)	17	14	2.6	54.0	56.6	4.6
		Autolyzed Pylorus				
20 c.c. egg yolk + 10 c.c. water extract	1	4	2.6	47.8	50.4	5.1
25 c.c. " " + 10 c.c. " "	4	6	3.5	Not saponified		
20 c.c. " " + 10 c.c. " "	6	6	3.05	"	"	
30 c.c. " " + 10 c.c. " " + membrane	8	6	2.8	47.3	50.1	5.5
20 c.c. egg yolk + 10 c.c. " " + membrane	15	5	3.7	5617	60.4	6.1
20 c.c. egg yolk + 10 c.c. " " + 10 c.c. glycerol extract	15	5	3.2	47.1	50.3	6.3
20 c.c. egg yolk + 10 c.c. glycerol extract of autolyzed pylorus	17	14	2.8	53.2	56.0	5.0

Table VII shows that the autolyzed mucous membrane has practically no ferment action, and it is to be assumed that the autolysis destroys the enzymes. This indicates the sensitiveness of the gastric lipase, considerably more sensitive than pepsin which even after the fundic autolysis showed considerable enzyme action. It is to be noted that the action of the autolyzed fundic and pyloric membranes is almost identical, which would strengthen the view that the enzyme is located in the fundic region of the stomach.

Table VIII

Glycerol Extract (Fundus)

	No. Days extracted	Length of Digestion hrs.	1st Titration Fatty Acids split by Enzyme	2nd Titration Fatty Acids split by saponification	Sum of Fatty Acids in 50 c.c. Ether	% of Fats split by Enzymes
20 c.c. egg yolk - 10 c.c. extract	1	18	2.2	48.4	51.0	4.3
20 c.c. egg yolk - 10 c.c. extract	2	15	3.4	46.7	50.0	6.7
20 c.c. egg yolk - 10 c.c. extract	4	15	3.8	Not saponified		
20 c.c. egg yolk - 10 c.c. extract	5	16	4.8	60.0	64.8	7.4
20 c.c. egg yolk - 10 c.c. extract	6	16	6.6	37.4	44.0	15.0
20 c.c. egg yolk - 10 c.c. extract	8	14	7.3	53.0	66.3	12.1
20 c.c. egg yolk- 10 c.c. extract	23	36	22.2	28.8	51.0	43.5

For the experiments shown in table VIII two stomachs of pigs were used and prepared as in table VI. They were preserved by adding thymol. The results are rather queer, for even after extracting for 5 days with glycerol no noticeable splitting had occurred. Fromme (1903) states that with the stomach of pigs he sometimes obtained negative results. So in order to further prove the lipolytic action of the stomach he experimented on dogs' stomachs.

The stomach from a dog recently dead from an experiment was chopped up and glycerol added in the ratio of 1:2. Thymol was next added, and the whole thing placed in an incubator. The acidity of the stomach, which was high, was reduced by the addition of tenth-normal sodium hydroxide. There still remained in the solution free hydrochloric acid. The solution was filtered. Twenty c.c. of egg yolk was treated on the water bath at 40° C. for six hours with 10 c.c. of this filtrate.

Table IX

	No. Days extracted	Length of Digestion hrs.	1st. Titration Fatty Acids split by Enzyme	2nd Titration Fatty Acids split by saponification	Sum of Fatty Acids in 50 c.c. Ether	% of Fats split by Enzymes
A		Gastric Juice of Dog				
10 c.c. of gastric juice filtered + 20 c.c. egg yolk	--	6	13.3	36.3	49.6	26.1
B.		Glycerol Extract I				
10 c.c. extract + 20 c.c. egg yolk	1/2	14	37.5	29.0	66.5	56.4
		2nd Glycerol Extract after 3 days of Previous Extraction.				
10 c.c. extract + 20 c.c. egg yolk	4	25	15.6	45.2	60.5	25.6
C.		3rd Glycerol Extract after 7 days of previous Extraction.				
10 c.c. extract + 20 c.c. egg yolk	16	36	8.5	41.2	49.7	17.3
		Glycerol Extract II				
10 c.c. extract + 20 c.c. egg yolk	1	15	28.7	25.2	53.9	53.2
10 c.c. extract + 20 c.c. egg yolk	2	15	21.8	26.0	48.4	44.1

In glycerol extract II the mucous membrane of the dog's stomach was placed on ice for 12 hours before extracting to see whether the enzyme is as easily destroyed as the one of the pig's stomach; but as can be see from table IX such is not the case. It did not lose any of its activity.

Volhard (1900-01) stated that the stomach contains a pro-enzyme or zymogen and an enzyme. He found that the enzyme of the glycerol extract is very sensitive to hydrochloric acid, while upon adding dilute alkali the fat splitting is accelerated. The reverse is true with the gastric juice; while alkalis destroy the enzymes, they are comparatively resistant to hydrochloric acid.

Fromme tested a dry powder, manufactured by Rhenania and Aachen from the mucous membrane of the pig's stomach, and found it very active. He added to the powder 15 c.c. tenth-normal hydrochloric acid and 20 c.c. of albumin plus 65 c.c. water; kept the mixture on the water bath for twenty-four hours at 40° C, and then filtered it. He found that the filtrate was practically inactive while the residue was very active.

Table X

Experiments with dry substance of Mucous Membrane of Pigs' Stomachs (Manufactured by Rhenania and Aachen)

A

	Length of Digestion hrs.	1st Titration Fatty Acids split by	2nd Titration Fatty Acids split by saponification	Sum of Fatty Acids in 50 c.c. Ether	% of Fats split by Enzyme
20 c.c. egg yolk + 2 g. dry substance shaken slightly	.6	5.75	23.8	29.3	19.4
20 c.c. egg yolk + 2 g. dry substance shaken 1/2 minute	6	11.4	31.2	42.6	26.7
20 c.c. egg yolk + 2 g. dry substance shaken 5 minutes	6	18.0	36.7	54.7	32.9
B. Five grams of dry substance + 100 c.c. glycerol, Rubbed together; filtered with difficulty.					
20 c.c. egg yolk + 15 c.c. filtrate (after 3 days)	5	1.6	44.8	46.4	3.3
20 c.c. egg yolk + 15 c.c. filtrate (after 3 more days)	14	2.9	51.9	53.9	3.7
20 c.c. egg yolk + 15 c.c. filtrate (after 5 more days)	18	1.9	50.5	52.4	3.6
C. Five grams dry substance + 15 c.c. N/10 HCl + 20 c.c. Albumin + 65 c.c. H_2O; let stand 24 hours on water bath at 40° C -- Filtered.					
20 c.c. egg yolk + 15 c.c. filtrate	20	2.6	45.3	47.9	5.4
20 c.c. egg yolk + residue	20	25.1	19.8	44.9	55.9

Table XI

	Length of Digestion hrs.	1st Titration of Fat split by Enzyme	2nd Titration Fatty Acids split by saponification	Sum of Fatty Acids in 50 c.c. Ether	% of Fats Split by Enzymes
A. 20 c.c. Glycerol Extract + 80 c.c. boiled Gastric Juice; Placed in incubator for 24 hrs.; filtered.					
50 c.c. filtrate + 20 c.c. egg yolk	18	3.3	65.7	69.0	4.8
20 c.c. Glycerol Extract + 20 c.c. of Albumin+ 60 c.c. H_2O; Placed in incubator for 24 hours; filtered					
30 c.c. filtrate + 20 c.c. egg yolk	18	1.8	41.8	43.6	4.1
B. Influence of alkali on the splitting of fat by Glycerol Extract.					
20 c.c. egg yolk + 5 c.c. extract	16	4.3	61.0	65.3	6.8
20 c.c. " " + 5 c.c. " + 2 c.c. N/10 NaOH	16	11.1	53.0	64.9	17.1
20 c.c. egg yolk + 5 c.c. " + 4 c.c. N/10 NaOH	16	14.6	54.8	69.4	21.0
20 c.c. egg yolk + 5 c.c. " + 6 c.c. N/10 NaOH	16	4.9	60.5	65.4	7.5
20 c.c. egg yolk + 10 c.c. fundic extract III + mucous membrane	16	6.6	37.4	44.0	15.0
20 c.c. egg yolk + 10 c.c. fundic glycerol extract III + mucous membrane + 2 N/10 NaOH	16	15.4	27.5	42.9	34.9
20 c.c. egg yolk + 10 c.c. fundic glycerol extract III + mucous membrane + 4 c.c. N/10 NaOH	16	16.9	26.7	43.0	38.7
C. Influence of alkali and acid on the fat splitting of the dry substance					
.5 g. dry substance + 20 c.c. egg yolk	24	9.1	39.0	48.1	18.7
.5 g. dry sub. + 20 c.c. egg yolk + 2 c.c. N/10 NaOH	24	35.2	12.0	47.2	74.6
.5 g. dry sub. + 20 c.c. egg yolk + 4 c.c. N/10 NaOH	24	29.0	13.6	42.6	68.0
.5 g. dry sub. + 20 c.c. egg yolk + 2 c.c. N/10 HCl	24	14.0	36.2	50.2	27.9
.5 g. dry sub. + 20 c.c. egg yolk + 4 c.c. N/10 HCl	24	6.2	41.0	47.2	13.1

Table XIa shows what was to be expected. The enzymatic action is practically nil, for boiling destroys the enzyme.

XIb shows very strikingly the favorable influence of alkali. This supports Volhard's contention (page 27). It further shows that the enzyme must be located in the mucous membrane of the fundic region.

XIc corroborates the above view and shows that the enzyme acts by far more favorably in the presence of alkali than in the presence of acid.

Fromme gives results to show that the gastric lipolytic enzyme of the dog's stomach differs from that of the pig's and approaches more nearly that of the human.

Table XII

The Different Behavior toward Alkalis and Acids of the Enzymes of the Pig's and the Dog's Stomach.

	Length of Digestion hrs.	1st Titration Fatty Acids split by Enzymes	2nd Titration Fatty Acids split by Saponification	Sum of Fatty Acids in 50 c.c. Ether	% of Fats split by Enzymes
A. Behavior of the Enzymes of the Pig's Stomach (III first Glycerol Extract.					
10 c.c. extract + 20 c.c. egg yolk	24	5.4	42.7	48.1	11.0
10 c.c. extract + 20 c.c. egg yolk + 1 cc. N/10 NaOH	24	10.8	38.7	49.5	21.8
10 c.c. extract + 20 c.c. egg yolk + 2 c.c. N/10 NaOH	24	13.6	35.4	49.0	27.7
10 c.c. extract + 20 c.c. egg yolk + 4 c.c. N/10 NaOH	24	15.7	35.1	50.8	30.9
10 c.c. extract + 20 c.c. egg yolk + 8 c.c. N/10 NaOH	24	5.5	47.7	53.2	16.3
10 c.c.extract + 20 c.c. egg yolk + 1 c.c. N/10 HCl	24	3.6	45.0	48.6	7.4
10 c.c extract + 20 c.c.egg yolk + 2 c.c. N/10 HCl	24	2.7	48.1	50.9	5.3
10 c.c. extract + 20 c.c. egg yolk + 4 c.c. N/10 HCl	24	2.2	49.2	51.4	4.3
10 c.c. extract + 20 c.c. egg yolk + 8 c.c. N/10 HCl	24	1.9	49.6	51.5	3.7

It can be seen from table XII that weak alkalis have a favorable influence on the lipolytic action of the gastric enzyme of pigs, while hydrochloric acid has a deteriorating effect.

The next table (XIII) shows the opposite to be true for the lipase of the dog's stomach. While with only slight amounts of alkali the lipolytic action is very effective, if the alkali is only slightly increased the action almost ceases; The opposite is true with the hydrochloric acid solution.

Table XIII

	Length of Digestion hrs.	1st Titration Fatty Acids split by Enzymes	2nd Titration Fatty Acids split by Saponification	Sum of Fatty Acids in 50 c.c. Ether	% of Fats split by Enzymes
Glycerol Extract of Dog's Stomach, I, diluted with Glycerol in the Ration of 1:2.					
10 c.c. extract - 20 c.c. egg yolk	12	18.7	25.5	44.2	42.3
10 c.c. extract - 20 c.c. egg yolk - 1 c.c. N/10 NaOH	12	19.5	25.7	45.2	43.1
10 c.c. extract - 20 c.c. egg yolk - 2 c.c. N/10 NaOH	12	20.2	25.6	45.8	44.1
10 c.c. extract - 20 c.c. egg yolk - 4 c.c. N/10 NaOH	12	1.2	17.6	18.8*	6.3
10 c.c. extract - 20 c.c. egg yolk - 8 c.c N/10 NaOH	12	0.8	12.8	13.6**	5.8
10 c.c. extract - 20 c.c. egg yolk - 1 c.c. N/10 HCl	12	19.0	26.9	45.9	41.4
10 c.c. extract - 20 c.c. egg yolk - 2 c.c. N/10 HCl	12	18.0	24.8	42.8	42.0
10 c.c. extract - 20 c.c. egg yolk - 4 c.c. N/10 HCl	12	13.0	17.3	30.5	43.2
10 c.c. extract - 20 c.c. egg yolk - 8 c.c. N/10 HCl	12	15.5	26.0	41.5	37.3

* 30 c.c. ether used
** 25 c.c. ether used

Ibrahim and Kopek (1909) experimented on children. Their method in short was as follows: newly born children who died, or foetus, were immediately dissected, the stomach taken out and the contents removed, and the inside washed thoroughly. The mucous membranes were scraped off with a dull scalpel and rubbed together with quartz sand. Pure glycerol was then added, also a few thymol crystals. This was well corked up and let stand for several days, either in an incubator at 38° C. or at room temperature. This glycerol extract was added to egg yolk emulsion and permitted to digest for a certain number of hours. The rest of the method was almost exactly like the one used by Zinnser (page 13). Control experiments were run.

Table XIV

Glycerol Extract of the Mucous Membrane of the Stomach

Number	Date	Age of Infant or Foetus	How long after death Dissected hours	Length of Body c./m.	Weight of Body grams.	Length of Extraction Days.	Condition of Extract	Length of Digestion hours.	First Titration c.c	Second titration c.c.	Sum two Titrations c.c.	% Fat split by Enzymes.	Remarks.
1	18/8/08	Embryo 4th month	11½	20	150	11	unboiled	22	1.0	22.7	23.7		Stomach and contents used.
2.	11/9/08	Embryo 5th Month	47	25	250	7	unboiled	21	1.3	23.0	24.3	0.6	Stomach and
							boiled	21	1.1	22.1	23.2		contents used.
3.	13/9/09	Premature birth 6th month	1/4	30	750	5	unboiled	35	1.9	12.8	14.7	7.5	Sieved 17 hours.
							boiled	35	0.8	14.0	14.8		Mucous membrane thoroughly washed and worked together with stomach walls.
4.	28/8/08	,,	15	31	780	9	unboiled	23	1.1	21.1	22.2	1.2	Twins
							boiled	23	0.9	23.1	24.0		Stomach and contents used.
5	,,	,,	15	31	800	9	unboiled	23	1.8	21.8	23.6	3.9	
6.	16/3/09	,,	15	33	760	15	unboiled	26½	3.9	26.2	30.1	9.3	Mucous membrane washed thoroughly and used together with stomach wall
					1100		boiled	26½	1.1	29.7	30.8		
7.	4/9/08	Premature birth 7th month	11 1/2	38	1100	10	unboiled	24	4.3	22.2	26.5	12.2	Stomach and content used
							boiled	24	1.1	26.3	27.4		
8.	,,	,,	4 3/4	38	1200	10	unboiled	24	4.4	24.7	29.1	11.1	Twins
							boiled	24	1.1	26.1	27.2		Lived 7 hours Mucous membrane washed slightly.
9.	16/3/09	Premature birth 8th month	13	42	1710	15	unboiled	26½	3.9	25.8	29.7	9.7	
							boiled	26½	1.0	28.5	29.5		Lived five days.
10.	25/3/09	Premature birth 9th month	8 1/2	46	2300	17	unboiled	23	5.2	21.7	27.9	19.0	
							boiled	23	0.9	26.5	27.4		
11.	8/9/08	,,	16	48	2150	8	unboiled	22	2.9	21.1	24.0	8.7	Lived ten hours.
							boiled	22	0.8	22.4	23.2		
12.	29/8/08	,,	9½	50	2080	9	unboiled	26	9.2	12.6	21.8	38.3	Stomach and
							boiled	26	1.0	24.3	25.3		Contents used.
13.	12/9/08	Newborn	3½	53	2800	5	unboiled	21	6.2	15.5	21.7	23.6	
							boiled	21	1.1	21.2	22.3		
14.	4/3/09	,,	10½	53	2850	35	unboiled	22½	5.8	19.5	25.3	18.8	Lived three days
							boiled	22½	1.0	23.4	24.4		Received, tea only.
15.	25/3/09	,,	25	55	4000	3	unboiled	23	5.4	22.6	28.0	15.6	
							boiled	23	1.0	25.9	26.9		
16.	12/9/09	,,	26	55	4550	3	unboiled	21	7.8	15.4	23.2	28.5	
							boiled	21	1.2	23.0	24.2		
17.	7/4/09	,,	27	56	4000	96	unboiled	48	7.1	10.3	17.4	36.9	Lived ¼ hour.
							boiled	48	0.7	16.6	17.3		

It is to be noted from table XIV that the glycerol extract of the mucous membrane of newly born children possesses considerable lipolytic power. It ranges from 15.6 to 36.9. Children born prematurely in the advanced months of pregnancy show similar results; and even those of a premature birth at the sixth month show a lipolytic activity of from 7.5 to 9.3. The only negative results were obtained with foetus 4 and 5 months old.

Number 17 of table XIV shows a very interesting case, that even after 96 days (at room temperature) the lipolytic activity was not diminished.

To prove the lipolytic activity of gastric juice, Ibrahim and Kopek took only such stomach contents as were similarly pure.

Table XV

	Date	Age of Infant or Foetus	Dissected after death hrs,	Weight of Body g.	Length of Body c.m.	Reaction of Stomach	Experiment	Condition of extract	Length of Digestion hrs.	2nd Titration	2nd Titration	Sum of Fatty acids	% of fat split by lipase	Remarks
1	8/9/06	Premature birth in 9th month	16½	2150	48	?	Extracted with glycerol for 8 days	unboiled	22	6.6	17.7	24.3	23.2	See table 14,
								boiled	22	0.9	21.9	22.8		
2	20/7/09	Newly born	15	2550	50	Alkaline	Added water directly	unboiled	22	10.8	6.0	16.8	58.9	
								boiled	22	1.0	16.3	17.3		
3	12/9/08	"	3½	2800	53	weakly acid	Extracted with glycerol for 4 days	unboiled	21	2.1	22.6	24.7	5.0	= Table 14, no.13
								boiled	21	0.9	25.0	25.9		
4	24/7/09	"	14½	2550	50	alkaline	Added water directly	unboiled	20½	6.9	7.5	14.4	41.9	
								boiled	20½	0.9	14.1	15.0		
5	24/7/09	"	36	3800	55	acid	Added water directly	unboiled	20½	4.6	8.9	13.5	39.4	
								boiled	20½	0.7	14.1	14.8		
6	22/3/09	"	25	4000	55	alkaline	Added water directly	unboiled	22	7.4	18.7	26.1	24.1	= Table 14
								boiled	22	1.1	24.6	25.7		No. 15
7	10/9/08	"	26	4550	55	acid	Extracted with glycerin for 1 day	unboiled	21	6.2	17.4	23.6	21.7	= Table 14,
								boiled	21	1.1	22.5	23.6		No. 11
8	23/7/09	"	36	4550	56	acid	Added water directly	unboiled	25	7.7	14.5	22.2	30.6	
								boiled	25	0.9	21.0	21.9		

Table XV shows without doubt that there is a great lipolytic power in the gastric juice of newly born children. The only inconsistency is number 3, where the splitting amounted to only 5 per cent. The reaction of the stomach does not seem to have much influence. It should be mentioned, however, that neither the alkaline nor the acid reaction of the gastric juice was very intense.

The question, still unsettled, was: Is the extracted lipase of the mucous membrane a product of the latter, or is it derived from the regurgitated pancreatic juice? To answer this question Ibrahim and Kopek (1909) tested for invertase in the stomach in three newly born children, and all gave negative results. This clearly indicated that there was no back-flow from the pancreas, otherwise the invertase should have been found in the stomach. (It should be noted here that Rietzel (1907) with a Pawlow fistula could not find any lipase, but he used toluol, and Ibrahim and Kopek showed conclusively that toluol inhibits lipase action in the stomach.

Regarding the question of fat absorption in the stomach there is a variance of opinions. The newer investigations, however, all point to the fact that fat is absorbed in the stomach.

Greene (1912) and Greene & Skaer (1913) have shown by histological methods that fat is absorbed in the stomachs of King salmon, Oncorhynchus Tschawytscha, and of cats and dogs. Greene States: "My observations show that gastric absorption of fat does not nearly equal the intestinal absorption in amount or volume. But the observations do show conclusively that the stom-

ach is a fat absorbing organ of no insignificant proportions."

Weiss (1912) showed that the epithelium of the stomach of the ring snake absorbed fat. The main absorption went on from the fundic to the pyloric region. There was, however, fat absorption also in the cardiac region of the stomach. He obtained similar results with young cats and dogs fed on olive oil or milk. He claims, however, that in cats and dogs the fatty droplets after a fatty meal can be observed in the epithelial cells of the stomach only when the cats and dogs are young. Greene takes exception to this.

Intestine

It is almost impossible to speak of digestion and absorption in the small intestine without at the same time discussing the bile and the pancreatic juice. I shall endeavor, however, to speak of the intestine as a separate independent organ, and shall discuss the bile and pancreatic juice later.

There is no question at present that the intestine is the most important organ of absorption. The only problem under consideration is, how is absorption carried on, and is there a special lipolytic enzyme, aside from the pancreatic enzyme, able to split fats, or may fat be absorbed as such without undergoing hydrolysis?

Falk (1914) claims to have found two enzymes in the duodenal juice, one more active toward tri-acetin than toward ethyl butyrate.

Connstein (1904) claims that there is no special lipolytic enzyme in the intestine. The fat is absorbed mainly in the form of fatty acids, glycerol, and soaps; in the epithelium it is again united in the form of neutral fat, and is carried as such in the chyle. In this he differs with many observers (Wells). The chyle fat is poured as neutral fat into the circulatory system.

Wilson (1906) concludes from microscopical studies of the condition of fat in the intestinal walls that "It is taken up in a particulate form by the striated border, either as neutral fat or fatty acids; in short, as an emulsion."

Croner (1909) claims that only the lower part of the lower part of the small intestine is able to absorb soaps, and

that neither bile nor gastric juice overcomes the inability of the upper part to absorb soaps. He also claims that emulsified neutral fat is absorbed through the whole intestine, altho most rapidly in the lower part.

Frauin (1907) found that if an emulsion of oil be introduced into an isolated loop of an intestine, after two hours it will be found that a certain quantity of free fatty acid is present in the lumen of the cells.

Lombroso (1904) states that when both pancreatic ducts were tied fat was yet absorbed to a large extent. The percentage of unabsorbed fat fluctuated from 8.1 to 78.8. By means of a villa fistula, he has shown that there was some lipolytic action in the intestinal canal upon almond oil.

In order to determine whether a dog whose pancreas was extirpated still had lipolytic action in his intestinal canal, three dogs whose ducts were tied were next operated on for a villa fistula, and then the pancreas was extirpated. Only one dog withstood the three operations and in this dog lipolytic action was quite distinct.

Argyris and Frank (1912-13) found that mono-glycerides are absorbed as readily as the tri-glycerides and more rapidly than fatty acids fed without glycerol. The results also support the view that fat must be split in the intestine before it can be absorbed, as can be seen from table XVI.

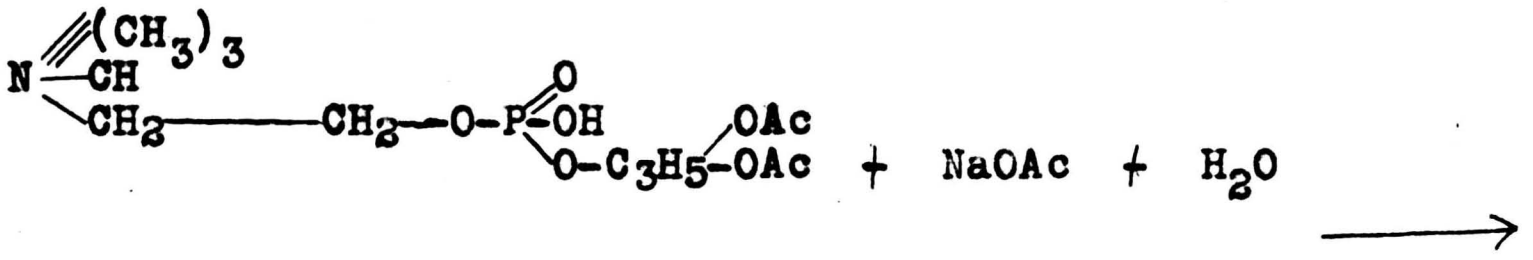
Table XVI

Food		Glycer-:ine	Saponi-fica-tion number	Acid number	Esterifi-cation number	Iodine number
I Mono-Olein						
*	determined	23.3	167.8	10.3	157.5	71.4
*	theoretical	25.8	---	----	157.6	70.8
	Chyle I	12.1	205.2	6.8	198.8	83.8
II Tri-Olein						
*	determined	11.8	203.5	3.0	200.5	90.1
*	theoretical	10.4	---	---	190.4	85.5
	Chyle I	11.9	202.2	5.1	197.1	85.7
III Mono-Glyceride						
*	determined	20.2	165.8	5.1	160.7	38.9
*	theoretical	26.0	---	---	160.0	---
	Chyle	11.5	196.4	8.6	187.8	48.6
IV Mono-Glyceride Chyle		11.6	204.4	7.9	196.0	49.7
V Hog's Fat						
*	determined	11.8	209.4	2.0	187.4	51.3
*	theoretical	11.0	---	---	190.0	---
	Chyle	12.5	205.0	8.0	197.0	52.0

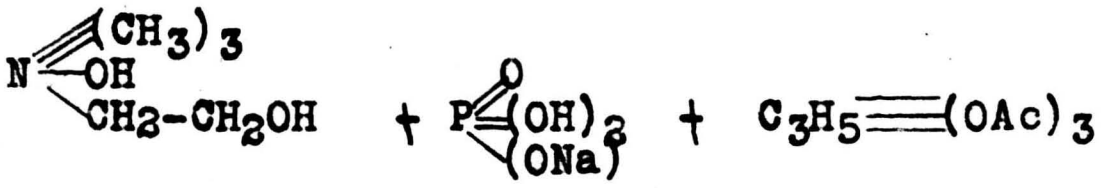
* Fat fed.

The mono-glycerides do not appear as such in the chyle, but are found as the tri-glycerides. This can best be seen from numbers IV and V of the experiment of table XVI, the results being almost identical. The only conclusion that can be drawn is that the mono-glycerides are synthesized to the tri-glycerides. This conclusion necessitates a previous splitting of the mono-glycerides. What happens to the extra glycerol has not been determined. It is also to be noted from the table that the iodine number of the chyle fat from tri-olein is lower than the original. Frank (1898) states that this may be due to the change of the food fat into body fat.

Frank, experimenting with the ethyl esters of the higher fatty acids, found that with the exception of the ester of stearic acid, the esters of the higher fatty acids were consumed in large amounts by a dog. Before they were absorbed they underwent cleavage in the small intestine; none of the esters were found in the chyle. The fat was synthesized to the tri-glyceride. In addition to the absorbed fat in the chyle, fatty material derived from the intestine or from the intestinal juices is also found. This secretion, however, is very small. The question whence the glycerol was derived was not solved. The author brings forth the hypothesis that it may be derived from lecethin, and gives the following equation



Lecithin Soap



Neurin Tri-glyceride

Boldyreff (1906-7) quotes Minkowsky and Hedon who found that dogs whose pancreas were extirpated were able to digest milk fat. Splitting of fat was also noted. It was supposed that the action was due to bacteria. Boldyreff concluded, however, that the action of the intestinal juice which is slow but sure must be due to a lipolytic enzyme. It acts very strongly on emulsified fat. Bile does not support the intestinal lipolytic action. The intestinal juice intensifies the pancreatic action. He further claims that the lower part of the small intestine is not able to split even emulsified fat.

Many contend that there are present in the intestinal juice albumins which are able to split the fat (Heidenhaus quoted by Boldyreff). Boldyreff claims, however, that its action is very small indeed, and besides there surely could not be very much albumin present in the small amount of intestinal juice used, from 0.5 to 1 c.c.

Cunningham (1898) claims that in the absence of both the bile and pancreatic juice from the intestine some absorption of fat, other than the naturally emulsified fat of milk, occurs.

London and Sivre (1909) claims that fats are absorbed mostly in the illeum.

London and Wersclowa (1908) state that cleavage occurs in the upper part of the duodenum, sometimes reaching an extent of 41 per cent before the intestinal contents meet with the pancreatic juice. In the small intestine cleavage reaches a maximum of 95 per cent.

Levites (1907) found that fat splitting gradually increases in the intestine until it reaches the coecum; and that the liberated fatty acids are mostly saponified into their sodium salts. The absorption begins in the small intestine and is almost completed when it reaches the coecum. He also found that the sodium salts are better absorbed than the fatty acids, and that glycerol fed to a dog by means of a jejuno-illeum fistula was largely absorbed before it reached the illeum.

Liver

The liver, besides its mere function of producing bile, holds a unique place in fat metabolism.

The fats occurring in the liver (also kidney and heart) differ in their properties from those found in the adipose connective tissue. The fats from the former yield on saponification a mixture of higher fatty acids which is far more complex than the mixture obtained from the connective tissue fat. The fat from the liver besides containing stearic, palmitic, and oleic acids, contains three higher unsaturated fatty acids of the series $C_NH_{2n-4}O_2$, $C_nH_{2n-6}O_2$, and $C_nH_{2n-8}O_2$ (Hartley 1909). Fats occurring in the liver are present not exclusively as simple glycerides as in the case of the fats of the adipose connective tissue, but to a considerable extent as more complex substances of the nature of lecithin, jecarin, cuarin, and a few others. The oleic acid found in the liver differs from other oleic acid. The double linkage in the liver oleic acid lies between the sixth and seventh carbon atoms, counting from the methyl group end of the chain. The double linkage in the oleic acid obtained from the adipose tissue is situated exactly in the middle of the chain (Hartley). Linoleic acid is present in liver tissue. Also an acid of the formula $C_{20}H_{32}O_2$ composing about ten per cent of the total fatty acids.

Leathes (1909) states that there is no other organ that is ever flooded with fat in the way that the liver is.

The appearance of large amounts of fats in an organ may be due to active production of fat in that organ, or else to impor-

tation from other parts.

Soxhlet (1881), Lawes and Gilbert (1883), very recently Leathes (1910) and a great many other experimenters found that fats are synthesized by animals mainly from the carbohydrates in the food.

Magnus Levy (1902) described a reaction occurring in the liver when portions of this organ were kept asceptically in a thermostat for some days. Not only acetic and lactic acids, but also butyric acid and a gas which was in part hydrogen was formed. The source of the butyric acid could not be determined positively, but there were some indications that it was derived from sugar. The simultaneous occurfence of hydrogen suggested an analogy with the butyric fermentation of sugar. Magnus Levy adopts as the explanation of the appearance of this butyric acid a hypothesis originally put forward by Nenki (quoted by Magnus Levy) which may be extended to account for the formation of higher fatty acids from carbohydrates. These observations, therefore, may be taken as pointing to the liver as a likely organ to which to assign the transformation of sugar into fat.

There is much ground for thinking that the large amounts of fat found in the liver are often due to the importation of fat from the storage places in the connective tissue.

Rosenfeld (1902) starved dogs for five days, and then fed them some phlorizin ($C_{21}H_{24}O_{10}$) on the sixth and seventh days. He found enormous quantities of fat in the liver amounting to as much as seventy per cent of the dry weight of the organ. He states that fat does not begin to accumulate until the fortieth hour after the first dose of phlorizin, and the animal is killed

eight hours later. According to this account , therefore, the enormous increase takes place in the course of eight hours. Again, if the animal be allowed to live another twenty-four hours without any further dose of phlorizin the liver is found to contain the normal amount of fat, recovery being as remarkably rapid as the development.

"It would be easier to understand such rapid changes as these if it were not necessary to suppose the fat to have been made where it is found." (Leathes, 1909).

Lebedoff and Rosenfeld (Leathes) have proved conclusively that the liver fat varies in character with the adipose fat.ue. The liver, therefore, seems to be the organ to which the migration of fat from other parts of the body occurs. In other organs the amount of fat may be increased 30 to 50 per cent, possibly 100 per cent above normal mean. In the liver the increase may be three to four hundred per cent above the normal mean, and even among normal animals something like one in ten or even five give figures that are fifty to one hundred per cent or even more above those which prevail among all the rest (Leathes, 1909). In fact, there is an extent of variation of fat in this organ compared with the variation of glycogen in this organ from the other organs of the body. But the interpretation is different in that the fat is imported from other parts of the body and not manufactured on the spot. The work of Hartley and Mavrogordath (1891) proves above contention that the fat of the liver comes from the adipose tissue. They have shown that the greater the percentage of fat in the liver, the nearer does the iodine value of that fat ap-

proach that of the iodine value of the normal fat of the adipose tissue. (The value of adipose tissue fat is about 70, while the normal iodine value of the liver fat is about 117).

The question is, how is the high iodine value of the liver fat explained. If the liver fat is derived from the adipose tissue, then there must occur a desaturation of the fats some where, since the acids stored away are more saturated than the fats in any of the organs in which we can suppose fat to be oxidized. Leathes states that it is probably due to the facts above described, that the liver is the organ where desaturation takes place, and the liver to a great extent prepares the fat in this way for other organs in which the oxidation is subsequently carried to a finish.

Dr. L. Meyer Weddel (Leathes) not only proves the above, but also proves that the character of the fat in the liver is determined not necessarily by the character of the fat which has been stored by the animal in weeks past, but by the character of the fat that has recently been circulating in the blood. This, of course, may in time of mobilization be fat from the stores of the adipose tissue.

She has fed cats and rats with oils of high iodine value, and the iodine value of the liver fat increased.

There is one thing that is difficult to explain, and that is why the iodine value of the liver fat is nearly always higher than that of the food fat. There are two general theories proposed (Leathes). One is "desaturation", and that may be merely a transposition of unsaturated linkages. We know that the properties of unsaturated fatty acids of the same empirical formula vary

according to the position of unsaturated linkage. For instance, the ordinary oleic acid has the double bond in the middle between the ninth and tenth carbon atoms. Le Seur prepared synthetically the acid with the double bond between the α and β carbon atoms and this acid would not decolorize bromine water. In certain positions therefore, the double linkage is more stable than in others.

The second theory or explanation of the high iodine value of the liver fat is given by Hartley (1907-08). Among the different hydroxy acids which he obtained, he found one which was very soluble in water and therefore must contain a large number of hydroxyl groups. The acid crystallizes well and melts sharply, and an analysis gives figures agreeing exactly with the Ortho-hydroxy arachidic acid ($C_{20}H_{32}O_2$). This indicates the presence in the liver of an acid which has a chain of twenty carbon atoms and four double links. He obtained the octo-bromide of that acid. He estimated that this acid is present in rather large amounts and constitutes about 8 or 10 per cent of the acids in the pig's liver.

Hartley and Mavrogordath (1891) showed that when these large quantities of fat occur in the liver the fatty acids are not those which normally characterize the fats and lipoids of the liver and other viscera, whose iodine value is about 130, but consist largely of the saturated acids and oleic acid, and therefore absorb in extreme cases hardly more iodine than the acids which can be obtained from the fat of adipose tissue.

Roper (1913) states "The fact that the liver often contains a large amount of fat (especially in abnormal conditions)

indicates that it is to this organ that the fat first comes when it is to be oxidized, since the other active organs of the body do not exhibit this fatty infiltration to anything like the same extent. When we examine the nature of the fat in the normal liver it is found to contain a much larger amount of unsaturated fats than that in the adipose tissue, and the question is, does this not represent a normal breakdown of fat in the liver for oxidizing purposes?"

Patton (1895) experimenting on dogs, found that the food fat influences the liver fat. Five kittens of a brood were suckled by their mother for about six weeks. They were then fed for two days on lean beef finely minced, and water.

From 11 A. M. on the 6th of August to 5:30 P. M. on the 7th of August they received no food. They then each received 50 c.c. of cream containing 18.7 per cent of fats, so that each received about 9 grams of fat.

A. was killed immediately. Scrapings of liver showed few oil globules of the cells, which stained dark brown with iodine. The stomach contained fluid cream.

B. was kept without food 16 hours, and then killed. The liver was pale and creamy, and scrapings appeared like an emulsion. The cells stained deeply with iodine. The stomach was empty.

C. was kept without food 28 hours. The liver was pale and creamy and contained many oil globules. The iodine staining was much less marked than in B. The stomach was empty.

D. was kept without food 48 hours. The liver was less pale than in B. and C. The cells contained numerous oil globules and stained only slightly with iodine. The stomach was empty.

E. was kept without food 72 hours. The liver was less pale than in B, C, or D. Only a few oil globules were seen in the cells. With iodine there was hardly any staining. The analysis of the liver gave the following:

Table XVII

Livers

	Wt. of liver	Solids	% of ether extract	% in liver	% of ether extract	Fatty Acids. % of solids	Melting points
A	13.0	25.0	3.57	1.71	47.6	6.84	45° C
B	16.6	23.4	9.09	6.23	68.5	26.6	34
C.	12.5	21.9	8.68	5.97	68.8	27.2	34
D	10.0	20.4	8.94	6.35	71.0	31.0	34
E	11.7	22.6	4.39	2.25	51.5	9.9	41

Melting point of fatty acids of cream 23° C

" " " " " " body 26° C

Three rats of the same litter were starved for 24 hours. On the evening of 2/9/94 B received 10 grams of beef fat. C received 7 grams of sugar with 14 grams of starch, while A was left without food. In the morning A was found dead. B and C were killed at 12 noon.

Table XVIII

	Wt. of Liver grams	Ether extract % of liver	Fatty acids % of liver	% of ether extract
A	1.6	3.56	1.69	47.3
B	2.0	5.85	3.55	60.6
C	4.0	3.42	1.52	44.5

It is plainly seen from this table that the liver of the rat which was given the beef fat contained much more ether soluble or fatty acids than that of either the starved rat A or the sugar fed C.

Bile

What part the bile plays in the digestion and absorption of fat has long been a subject of much discussion. It has, however, been proved conclusively that the bile greatly assists in the digestion and absorption of fat, and by its absence absorption is greatly diminished. Many investigators (Pflüger, 1900) supposed that the bile acts as a solvent for the soaps and fatty acids. Others, that it activates the pancreatic lipase (Howell, 1911); still others, that it assists in the emulsification of the neutral fats. Many attributed its activity to its lipolytic action. It shall be my purpose here to review some of the literature regarding the activity of the bile and the causes of that activity.

Röhman (1882) states that if bile be excluded only about 40 to 50 per cent of the fat will be absorbed. Hewlett (1905) concluded that the accelerating action of the bile salts on the lower fatty acids was due to lecithin contamination. This conclusion was opposed to the generally accepted view that the bile salts are the important constituents of the bile in assisting in the absorption of fat (Howell, 1911).

Loevenhart and Souder (1906) found that bile salts, lecithin, and bile greatly accelerate the action of pancreatic juice, and that the combined effect of the bile salts and lecithin is but very little greater than that of bile salts alone. They

further found that the effects of bile salts, and bile of a corresponding concentration of bile salts, very closely approximate one another.

Pflüger (1901-02) states that the solution of fatty acids in the intestines is accomplished by the combined action of bile, sodium carbonate, and the soaps.

Hamsik (1910) doubts whether soaps are taken up as such. That soaps hydrolize in the presence of living intestinal epithelium has been shown by Moore.

Croner (1909-10) investigated various parts of the intestine and stated that only the lower parts of the intestine absorb soap. Hamsik states that bile, besides its solvent power upon fatty acids and soaps, helps fat synthesis by the pancreatic and intestinal lipase.

Rochaix (1912) states that elimination of the bile from the intestinal tract markedly increases the fat excreted.

Rockford (1891) states, "If bile be present an emulsion can not form. This has been explained by the fact that the soap-dissolving properties of the bile prevented the formation of insoluble soap membranes, and that the unprotected oil globules ran together and came to the surface as free oil."

The pancreatic juice acting in the presence of an equal quantity of a mixture of 0.25 per cent solution of hydrochloric acid and bile will require one-fourth the time it would take the pancreatic juice alone. It seems, therefore, that the mixture of percentages of hydrochloric acid found in the duodenum and of sodium carbonate in the bile expedite the fat splitting action of the pancreatic juice. The bile and hydrochloric acid absolutely

preclude the formation of emulsions. The duodenum, therefore, offers the most favorable conditions for the splitting of the fats and the most unfavorable for their emulsification. In the jejunum and illeum these conditions seem to be exactly reversed. The intestinal juice containing, as it does, 0.25 per cent sodium carbonate would not only furnish the conditions for the spontaneous emulsification of the fatty acids but would also retard the fat splitting action of the pancreatic juice. Bile alone does not split fats (Rochford)

Wohlegemuth (1907) found that bile possessed lipolytic activity, but it was not the lipase of the bile which assisted the pancreatic juice in the splitting of fat, but the glycocholic and taurocholic acids.

Pancreas

That the pancreas has a great influence on fat absorption has been recognized more or less since the time of Claude Bernard (1846). But there is a difference of opinion as to how this is accomplished. Most investigators ascribe the influence to a definite lipase, others to its emulsifying power, and still others claim that the pancreas has an internal function, but what the latter is has not yet been found out. There are several investigators who claim that even if all the pancreatic juice be excluded from the intestine normal absorption occurs.

Visentini (1910) states that if the pancreatic secretion is not allowed to flow into the intestine such disturbances follow as are usually associated with pancreas extirpation, e.g., a decrease in fat absorption

Pratt, Lamson, and Marks (1909), who studied on dogs, state "In every instance in which the pancreatic secretion was excluded from the intestine there was marked diminution in the absorption of fat. The lack of absorption was not due to the absence of a fat splitting enzyme, for the proportion of split fat in the feces was normal. The disturbance in metabolism did not develop gradually, but was noted as soon as the animal recovered from the operation. It persisted as long as the animals were under observation which in one case was five months and in another four months. The animals lost weight. Additional evidence that the loss of fat in the feces was caused by lack of the external pancreatic secretion is shown by the increase in absorption when pancreatic ferments were given by the mouth."

Nieman (quoted by Lombroso) tied the pancreatic ducts

and from his observations came to the conclusion that the absence of pancreatic juice from the intestine produced no disturbance in absorption.

Burkhard (1908) on the contrary, pointed out that the external secretion of the pancreas is essential for normal absorption of fat.

Boldyreff (1906) states that while bile promotes the digestion and absorption of fat, it does not play an independent role, so that in case the pancrease is in poor working order or is entirely extirpated the body can not make use of the food fat.

Rochford (1891) states that the pancreatic juice was assumed to have two functions; first, that of causing an emulsion, and second, that of splitting neutral fat into acid and glycerol. "These two processes are now associated together, and the accepted view is that the emulsion is wholly due to the fatty acids which had been developed in the oil by the fat splitting ferment."

The action of pancreatic juice on most fats is rapid and complete (castor oil a notable exception, a fact which may account for its laxative properties.)

Pancreatic juice acts slowly on fats which have a melting point above body temperature; however, their solidity at body temperature does not prevent their being split (Rochford).

Colin (quoted by Hartley) stated that the absorption of fat remained undisturbed when the pancreatic juice was allowed to escape from a fistula. Harley (1895) states, however, that in dogs which have their pancrease removed the quantity of fat given is not only again recovered, but a surplus is found, which

is probably derived from the intestinal secretions or excretions. The power of passage of fat from the stomach is very much delayed by the extirpation of the pancreas.

Lombroso (1908-09) agrees with Harley that when the pancreas is extirpated more fat than is fed is excreted.

Table XIX

Wt. and kind of animal	Date of pancreas extirpation	No. of days of fat excretion	Amt. of fat fed	Amt. of fat excreted	No. of days after tying and cutting the pancreatic duct
6.8 K°. Bitch	10/11/02	10/13-18	80 g. of beef fat	104	81
5.5 K°. Bitch	5/27/03	5/28-6/2	133 g. (33 g. horse fat and 100 g. of beef fat	151	56
8.8K°. Dog	2/23/04	2/24-28	104 g.	128	30
"	"	2/28-3/2	18 g.	90	"

It is to be noted that the animal was not given any food twenty four hours before pancreas extirpation. It was also given sulfur as a laxative. Furthermore, several days previous to that it was only fed bread and egg albumin.

Table XX

Wt. and kind of animal	Date of pancreas extirpation	Amt.of fat fed gms.	Amt. of fat excreted	Addition of pancreas or juice	No.days of fat excretion.	No. days after tying and cutting the pancreatic duct.
6.1 K. Bitch	9/4/02	145	118.6	+	9/27-10/4	100
"	"	90	110.9	---	10/4-9	100
"	"	304 (milk)	152		10/15-11/1	100
"	"	"	241		11/1-15	100
8.3 K. Dog	7/27/02	95	68.8	Pancreatic juice placed in duodenum	8/9-12	19
"	"	95	105.2	-----	8/12-15	19
"	"	115	127.1	-----	8/15-19	19

It can be noted from this table that the addition of the pancreatic juice only slightly diminishes the amount of fat excreted. This contradicts Pratt, Lamson, and Marks (page 56).

Lombroso also injected pancreatic juice into the circulatory system, but the amount of excreted fat did not diminish any.

In order to find out whether or not the excreted fat contains all the food fat other experiments were made as shown in the next table.

Table XXI

Wt. and kind of animal K°.	Date of pancreas extirpation	Amt. of fat fed	Amt. of fat excreted	No. of days of fat excretion	No. of days after tying and cutting the pancreatic duct	Melting point of fat excreted
5.5 dog	5/20/04	87 g. (70 gms. of oil of sweet almonds and 17 gms. fat in horse flesh)	79	May 21-25	20	29-30° C.
7.1 Dog	6/23/04	104.9 (100 gms. oil of sweet almonds; 4.9 gms. in bread and protein	98	June 25-29	30	22-25° C.

It is to be seen that the excreted fat had a higher melting point than the fat fed. This phenomenon has been noted in normal animals, and it was assumed that in the process of absorption in the intestinal canal the fats of lower melting points are selected, so to speak (L. Zoia quoted by Lombroso). This was not shown, however, in Lombroso's experiment; first, because the oil of sweet almonds has a very low melting point, second, because the amount excreted was always higher than the intake, and so surely the change could not come from the food fat.

To test whether the sole purpose of the pancreas is to split neutral fat he experimented by feeding either fatty acids or soaps.

Table XXII

Wt. and kind of animal K°.	Date of Pancreas extirpation	Amt. of fat fed as fatty acids	Amt. of fat excreted	No. of days of fat excretion	No. days after tying and cutting the pancreatic duct	% of fed fat to excreted fat
6.1 Bitch	9/4/02	200 g. (150 g. beef fat , 50 g. of fat in horse flesh	182.39	9/9-14	100	88.8
"	"	71 g. (56 g. oleic acid, 15 g. fat in horse flesh	71.55	9/14-18	100	100.7
"	"	62 g. (52 g. of NaOleate & 12 g. fat in bread & milk	58.1	9/18-22	100	93.69
7.1 Dog	6/23/04	104.9 g. (101 g. oil of sweet almonds, & 4.9 g. fat in bread and protein	98	6/25-29	30	93.30
"	"	126 g. (120 g. pure oleic acid, 6 g. fat in bread & protein.	118.8	6/29-7/4	30	94.20

there is a slight diminution in the amount of excreted fat but not enough to account for the usual assumption that the purpose of the pancreas is only to split fats.

Table XXIII

Wt. & kind of animal	Date of extirpation	Amt. of fat fed	Amt. of fat excreted	No. of days of fat excretion
8.2 K°. Dog	Feb. 24/04 (80 gms. of beef fat and 24 gms. of fat in horse flesh	104 gms.	128 gms.	Feb. 24-28
"	"	18 gms.	91 gms.	Feb. 29-Mch. 3.

On March 3 at 10 A. M. 30 grams of pure oleic acid was fed to the dog by means of an opening in the stomach. At 3:30 P. M. the dog was bled to death. A histological examination of the intestinal epithelium showed that there were fatty droplets as in a normal dog, which indicated absorption. These results show that some fats are absorbed even in a dog without a pancreas. Therefore a great amount of excreted fat must come from the body fat. It seems that both these amounts are equal(Lombroso, 1909).

Lombroso admitted the pancreatic secretion of one dog into another and was able to diminish only slightly the amount of excreted fat.

It has been suggested that on extirpation of the pancreas there usually arises a state of glycosuria, and that may hinder fat absorption; but Lombroso denies that, for in one case he was able to prevent glycosuria and the absorption did not improve much. He therefore came to the conclusion that the pancreas possesses an internal function whose presence is necessary in order that fat stored up in the tissues may be rendered available for the organism. This assumption of an inner function of the pancreas is opposed by Burkhard (see page 57).

Hochford (1891) with fresh juice of rabbit pancreas was able to completely decompose all fats except castor oil. The latter, however, differs greatly chemically and pharmacologically from all other oils (see page 57).

Müler (Connstein 1904) found that in a mixture of milk in the presence of the pancreas of a pig in a slightly alkaline soda solution during a period of 24 hours 86.4 per cent of the fat was split.

Pawlow and Walther (Connstein 1904) showed that not only does fat excite pancreatic secretion, but that the latter is reacher in lipolytic enźymes (see curves on page). In the first two hours after feeding milk the pancreatic secretion is extremely rich in lipase. If the milk be made fat free there is no change in the flow nor in the amount of secretion, but a diminution in the amount of pancreatic ferment.

Belina (1913) states that neutral fats are intense stim-

ulants of the secretion of pancreatic juice. After the administration of fat the pancreatic juice contains its largest proportion of lipase. Fatty acids and soaps produce similar effects.

Babkin and Ishikawa (1913) state that while it is possible that the neutral fats are starters of pancreatic secretion, fatty acids and soaps are the main excitors. Connheim and Klee (1913) found that oil in the duodenum did not excite any more secretion than did water.

Table XXIV

Introduction of 50 c.c. of neutral Poppy Oil into The Stomach.

October 12, 1911

Time	Amt. of Pancreatic secretion in c.cm.		Remarks
10:00-10:20	0.3 c		Reaction in stomach alkaline
At 10:20 50 c.c. neutral poppy oil was introduced			
10:20-10:35	0.1	Total	At 10:37 there appeared in the
10:50	0.9	3.7	duodenum a clear oil. The intes-
11:05	1.7		tinal fistula closed.
11:20	1:0		At 10:42 there was noted an ac-
			celeration of pancreatic secretion.
11:20-11:35	2.6		At 10:45 the contents of the
11:50	2.2	Total	duodenal fistula were colored
12:05	1.5	7.1	with bile. At 11:00 bile re-
12:20	0.8		gurgitated into the stomach.
12:20-12:35	0.8		At 12:35 a mixture of 45 c.c. of
Total in 2 1/2 hours	11.6		oil and juices of a strong acid reaction was let out of the stomach.

Table XXV

Introduction of 100 c.c. of neutral Poppy Oil into the Stomach.

November 19, 1911.

Time	Amt. of pancreatic secretion in c.cm.		Remarks
10:00-11:05	0.3		Stomach weakly alkaline
11:20	0.3		
At 11:21 100 c.c. of neutral poppy oil was introduced			
11:20-11:35	2.6		At 11:23 there appeared in the
11:50	5.1	Total	duodenum a clear oil. The intesti-
12:05	2.8	12.3	nal fistula closed. At 11:27
12:20	1.8		pancreatic secretion began.
			At 11:40 the contents of the duo-
			denal fistula were colored with
			bile. At 12:12 bile regurgitated
			in the stomach and the juice became
			acid.
12:20-12:35	1.5		
12:50	1.0	Total	
1:05	1.3	5.0	
1:20	1.2		
1:20-1:35	1.5		1:35 a mixture of 40 c.c. of fat
			and different juices of a strong
Total in 3 1/4 hours	18.8c.c.		acid reaction was let out of the
			stomach.

Table XXVI

Washing the Duodenum with a 2 per cent Solution of Sodium Oleate.

January 3, 1912

Time	Amt. of pancreatic juice in c.c.		Amt. of soaps in the duodenal fistula in c.c.	Remarks
12:20-12:35	0.2			Reaction of stomach
12:50	0.1			alkaline
At 12:50, 100 c.c. of a 2 per cent sodium oleate was admitted into the stomach				
12:50-12:55	0	Total	4.0	At 12:53 soap was noted in
1:00	0	1 drop	16.0	the duodenal fistula.
1:05	1 drop		5.0	Pancreatic sectretion began 1:04
1:05-1:10	0.4	Total	18.0	At 1:10 there was 55 c.c.
1:15	0.2	0.7	24.5	of fluid in the stomach of
1:20	0.1			alkaline reaction
1:20-1:25	0.5	Total	5.5	At 1:21 there was 10 c.c.
1:30	0.4	1.1	a few	of fluid of alkaline reac-
1:35	0.2		drops	tion in the stomach (It was not poured back into the stomach)
				1:30 reaction in stomach alk.
				1:35 " " " "
Total in 3/4 hour		18.0	102.5	There was no bile produced.

Table XXVII

Pouring into the Duodenum a 0.25 per cent Solution of HCl.

Time	Amt. of pancreatic juice in c.c.		Amt. of HCl in the duodenal fistula in c.c.		Remarks
Before pouring in the acid, there was admitted into the duodenum at 10:45 o'clock 25 c.c. of neutral oil. When the pancreatic secretion seemed to be at an end 100 c.c. of the acid was placed in the stomach--The intestinal fistula opened--reaction in duodenum, neutral					
12:00-12:05	0.3		9.0		From 12:02 to 12:44 the fluid coming thru the fistula was mixed with bile, and was either neutral or weakly alkaline.
12:10	0.2	1.0	1.0		
12:15	0.5		2.0		
12:15-12:20	0.4		1.0		
12:25	0.3	1.0	1.0		
12:30	0.3		0.0		
12:30-12:35	0.3		0.0		
12:40	0.3	1.1	0.5		
12:45	0.5		6.5		After 12:44 the flow of HCl was rapid
12:45-12:50	1.2		19.0		
12:55	4.0	8.4	25.0		
1:00	3.2		17.0		
				93.5	
1:00-1:05	3.8		15.0		At 1:15 a greenly colored sour fluid was let out of the stomach
1:10	2.4		10.0		
1:15	2.2		1.0		

The tables of Babkin and Ishikawa show that acids have a great excitory action on pancreatic secretion. This shows, most probably, that the secretion of the pancreatic juice once started would, by the action of the split fat, excite the secretion of more juice until all of the fat would be split and absorbed.

We have seen that Babkin and Ishikawa as well as Müler found that fat excites pancreatic secretion and that secretion is rich in lipase. This differs from Bradley (1909) who found that with human pancreatic juice there was no definite relation between diet and enzyme action.

Wohlgemuth investigated the secretion of the normal human pancreas of a boy. An accident occurred to the latter which made an opening thru the abdominal wall and when he began to convalesce the pancreatic juice was studied by means of a fistula. It was found that after feeding cream or milk the flow of pancreatic juice was far less than after feeding carbohydrate or protein foods.

Table XXVIII

Corresponding to Curve I

	1st hr.	2d hr.	3d hr.	4th hr.	total
Carbohydrates	30	26	15	14	75
Protein	17	18	17	14	66
Fat	7	6	12	10	35

Wohlgemuth also determined the concentration of the different juices. Curves 2, 3, and 4 show the different concentrations. Curve 2 gives the concentration of the pancreatic

juice when protein was fed. Curve 3 represents the concentration of the juice when carbohydrate was fed, and curve 4 represents concentration of juice when fat was fed. Examining the curves we find that the fat juice concentration is the highest of the three. Comparing these concentrations with the amount of juice produced, we find the concentration varies inversely as the amount, the greater the amount of juice produced the less the concentration and vice-versa. This principle can also be illustrated by the following table which is figured on the average of the concentrations of the several enzymes.

Table XXIX

Food	Total Juice	Average concentration of Trypsin	Diastase	Lipase
Carbohydrates	75	5.3	4.8	325.0
Proteins	66	10.89	6.25	529.0
Fats	35	14.97	10.89	954.8

Babkin and Ishikawa, experimenting on a dog, came to the same conclusion, that is, the concentration varies inversely as the amount of juice produced.

Fromme (see page 30) found that there exists a similarity between the pancreas of the human and the dog as regards acids and alkalis. Wohlgemuth shows similar results, as is beautifully illustrated in curve 5. With hydrochloric acid the greatest amount of juice was obtained in the first half hour, and this fell back in the second half hour. Alkali, on the other hand, is almost normal up to the second half hour, then falls considerably and does not reach normality even after the fourth half hour. This knowledge of the effect of acids and alkalis becomes very in-

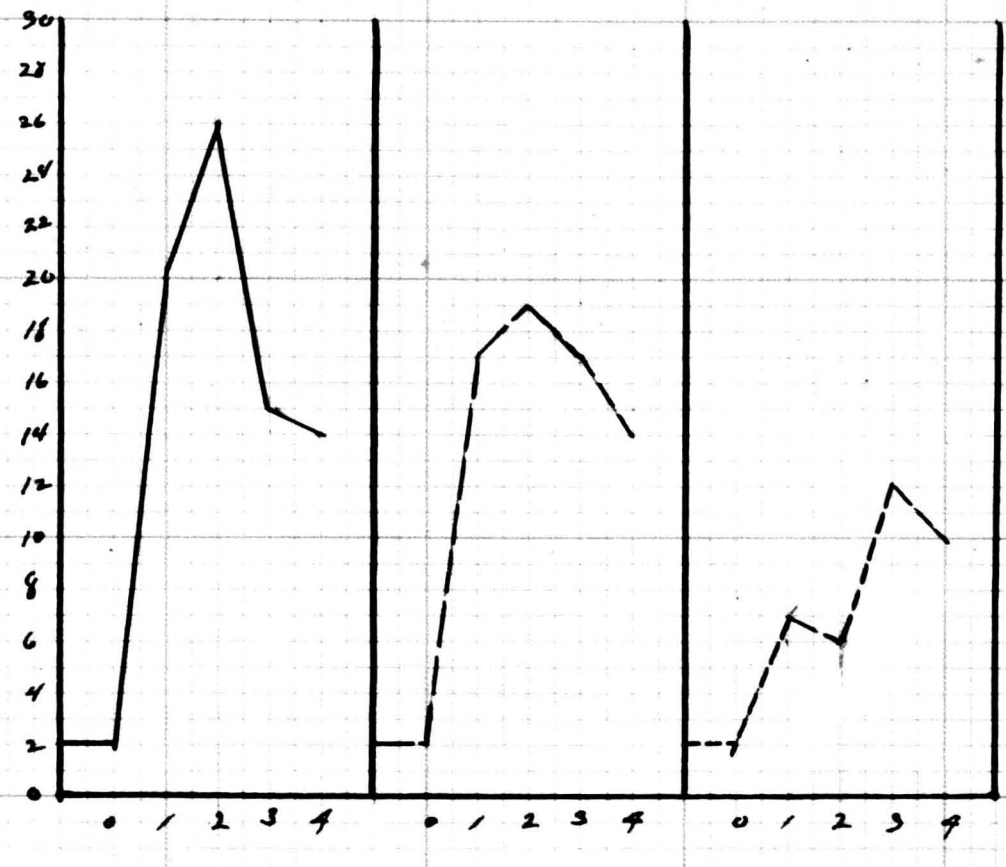
teresting when we ask ourselves, what is the effect of the hydrochloric acid of the stomach on the flow of pancreatic juice?

Pawlow (Wohlgemuth 1907) maintains that when the hydrochloric acid enters the duodenum it exerts a reflecting stimulus on the pancreas directly. Bayliss and Starling (Wohlgemuth) and Henry (1910),on the other hand, think that the hydrochloric acid extracts from the mucous membrane a substance which they call secretin. This substance after entering the circulatory system or, in other words, after absorption excites the pancreatic secretion.

As far as we know most of the investigators do not mention the above assumption, and consequently there is not much data available to substantiate the hypothesis.

C = Carbohydrates
P. = Protein
F = Fat.

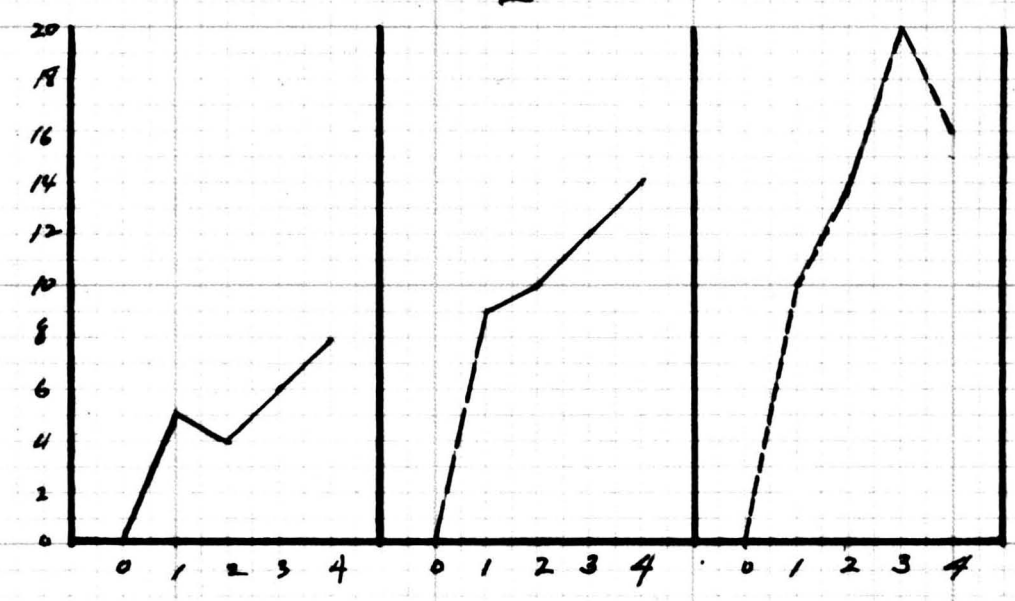
1



Curve 1

Ordinates = c.c of juice
Abscissas = hours.

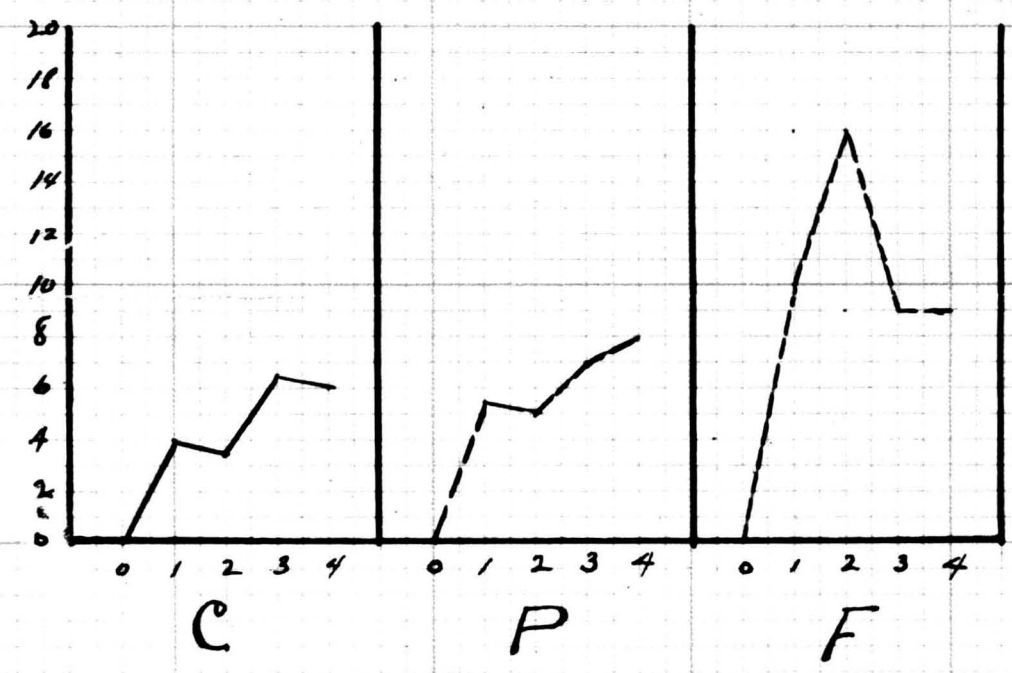
2



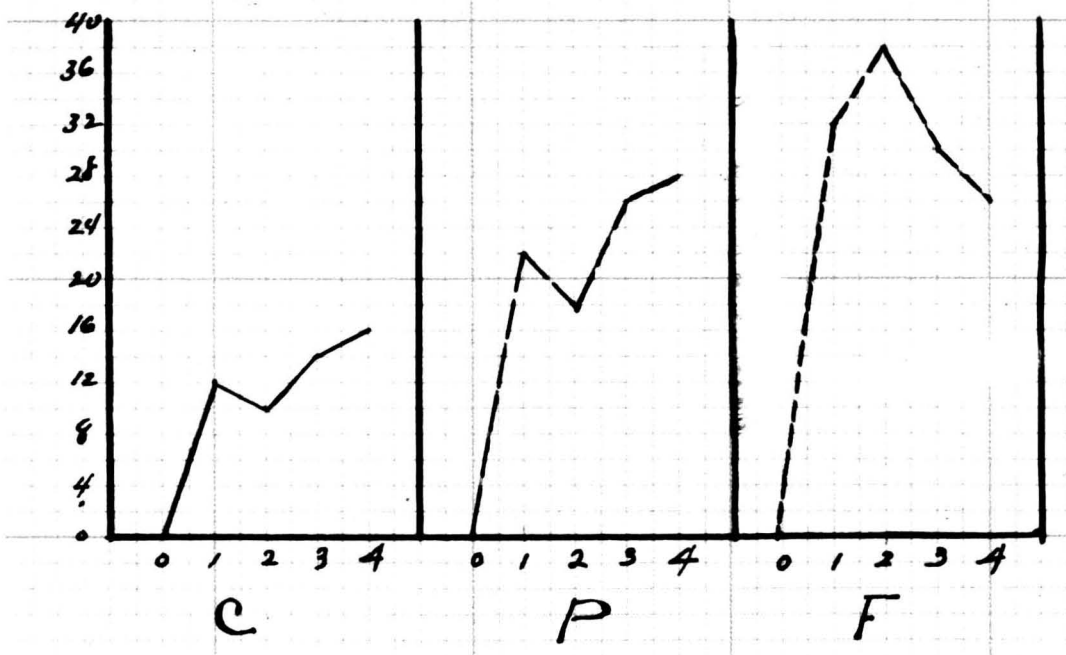
Curves 2 and 3

Ordinates = cubic m.m of column of starch
Abscissas = hours.

3.



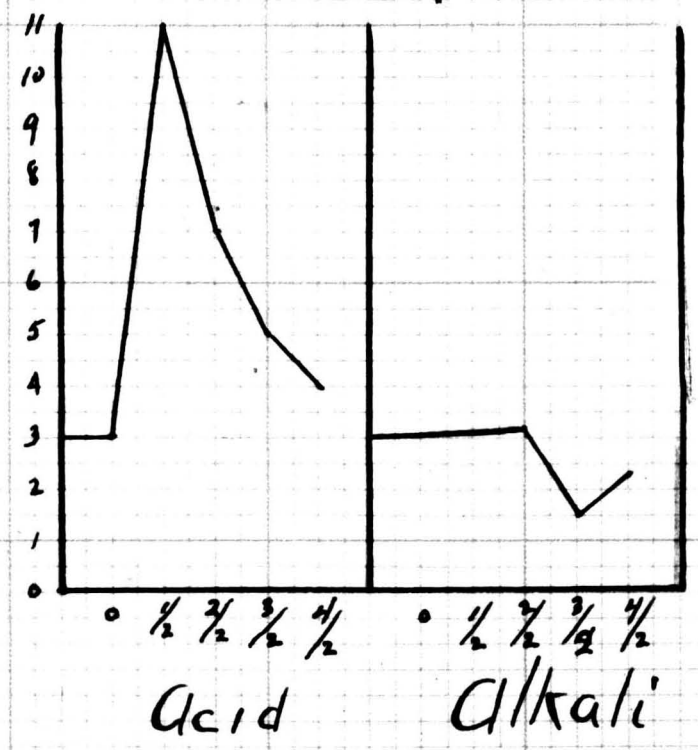
4.



Curve 4

Ordinates = cc. tenth-Normal Sodium hydroxide
Abscissas = hours

5.



Curve 5

Ordinates = c.c. juice
Abscissas = hours.

Part III

Absorption

I have given a historical account of the organs of the body that are most important in the absorption of fat. I have not, however, given any attention to the question as to how the fat passes into the body and is utilized by the different organs. In order to make the subject more intelligent and complete I shall present in a few paragraphs the principal theories regarding the same.

There are two theories as to how fat is absorbed. The more generally accepted one is that the fat is absorbed by means of the lymphatics. The other is that the fat may also be absorbed by means of the blood vessels directly. (Patton 1895).

Fekete (1911) concludes that fat is absorbed in solution only and by means of the lymphatics.

Assuming that the latter is the correct theory, the question is: after the epithelial cells take up the fatty acids, then what becomes of them? Noll states that there is a synthesis of fat in the epithelial cells, as he has proved in the case of rats. This agrees with the work of Munk (1902) who states that it is impossible to say at present how much or how little fat is absorbed in an emulsified form.

Many observers state that fat may be absorbed as such. Several investigators, however, state that only fatty drops can be noted in the farthest inner part of the epithelial cells and they conclude that fat may only be absorbed as soaps; which are then transformed into neutral fat and fatty acids. Munk concludes that fatty acids during absorption combine with glycerol, and the fat appears as neutral fat in the lymph ducts. He

considers the combination with glycerol as very important, especially when the change of soaps into neutral fats is concerned, for the soaps are very poisonous when introduced into the circulatory system.

Noll (1910) is undecided about the chyle fats while he claims that he is almost certain that they are derived mainly from the fat of the epithelial cells. Like the latter fat, it also does not contain free fatty acids. The chyle fat, however, does not seem to have the same properties as the epithelial fat, e.g., it does not blacken on contact with osmic acid. He does not seem to think, however, that the reason why the chyle fat does not blacken with osmic acid may be due to a chemical change, but he would attribute it to a physical property because he asserts that the chyle fat is in a state of very fine emulsion, and henceforth will not darken on contact with osmic acid.

Noll, like Patton, also comes to the conclusion that the chyle ducts may not be the only means of transporting fat from the intestinal wall. He claims that it is probable that the blood vessels take a part in transportation. He comes to the above conclusion because, when feeding fat to a rabbit, he was able to detect fat in the lymphatic duct only after the fifth hour of digestion in spite of the fact that the epithelial cells already contained much fat. So, according to him, it is possible that the fats are absorbed thru a different route, and only go thru the lymphatic duct after they have overstepped the limit.

Noll comes to the conclusion, as many others have done, that the microscopic manner of detecting fat is insufficient.

I. Munk (1900) argues that since fats of lower melting point are better absorbed than those of higher (Arnschink, quoted by Munk) he must come to the conclusion that fats are largely absorbed as such, for otherwise what would the melting point have to do with absorption since, as he says, saponification is entirely independent of melting point. According to his idea, then, fats would not change at all during the whole process of absorption. He states that to assume that fats are absorbed as soaps or fatty acids and again resynthesized in the deeper portions of the epithelium would be an unnecessary supposition.

Moore (1904) claims that fatty acids and fats have the same appearance in the basal parts of the epithelial cells of the small intestine after appropriate staining. Therefore the method commonly employed does not show conclusively that fats are split into fatty acids and glycerol and absorbed as such. Chemical studies which he carried on, however, are believed to have established the correctness of the above theory. Washed and scraped mucous membrane obtained from a digesting animal contained 15 to 25 per cent of its fat in the form of fatty acids, while in the fluid of the lacteals 96 per cent of the fatty material was present as neutral fat. "The synthesis therefore was completed in the mucous membrane of the intestine."

Hoffbauer (1900) tried to prove that fat can be absorbed in an emulsified form without being previously hydrolyzed. He fed fat in which he used Alcanna red and Lacroth A, and showed that although these pigments are insoluble in water the fat appears in the thoracic duct colored red. By this he tried to contradict Pflüger who stated that fat can only be absorbed in so-

lution. Pflüger (1900), however, shows that these coloring materials are soluble in soaps, bile, glycerol, and therefore Hoffbauer's conclusions are incorrect.

Bloor (1912) states that it is generally accepted that under normal intestinal conditions most if not all the food fat is saponified in the intestine before absorption, and is absorbed as soaps. But since Munk (see page 74) has pointed out that soaps are very poisonous when injected into the circulatory system, we must conclude that the soaps are resynthesized to neutral fat before the latter is taken to the fat depots.

The Blood

The fat is discharged from the lymphatic ducts into the blood, and is carried by the blood to the different organs and to the fat depbsits. How the fat is carried in the blood is yet an undecided factor. It seems probable, however, that it is a mixture of neutral fat and fat in combination with cholesterol and other unsaponifiable substances.

Many investigators tried to find out whether the food fat influences the fat content of the blood. The trouble, however, was that we have not an exact method of determining fat and especially fat of blood.

Newman (1907) states that by means of a Heichert Ultra-condenser attached to an ordinary microscope he was able to discern fat droplets in his own blood and in the blood of some of his colleagues after a meal containing fair amounts of fats. When he examined his blood in the morning between 8 and 11 on an empty stomach, or when he ate a meal not containing fat, these droplets

were not present. If he ate a breakfast rich in butter fat he found the number of these droplets greatly increased (5 hours later).

He ate breakfast consisting of lean meat and tea and while these droplets increased, they were only very slight and may have been dur to the amount of fat contained in the lean meat, 1.3 grams.

Connstein and Michaels (1898) state that when they added chyle fat to the blood it disappeared. The fatty acids which should have been obtained in its place (due to lipolytic action) could not be found. This agrees very well with the statement of Hürthle (1895) who claimed that the fat is combined in the blood with cholesterol to form the latter soap, which statement also agrees more or less with that of Abderhalden (1908) who said that blood possesses the ability to transform fats into unknown substances which are soluble in water and capable of dialysis.

Abderhalden and Lampe (1913) state that the greater part of the fat of a fatty meal undoubtedly remains only a very short time in the blood before it is removed.

Part IV

Fat Splitting Enzymes or Lipase

I have spoken in the previous pages concerning the action of splitting enzymes or as they are better known under the name of lipase. We have considered the arguments concerning their activity. However, it is of the utmost importance to know how they act and how widespread is their action to account for all the changes going on in fat in the animal organism.

A great amount of work on lipase action has been done by Kastle and Loevenhart. They, however, are by no means the only investigators in this field. The action of the lipase is like the ordinary hydrolytic process of fat splitting. If N represents the neutral fat, F fatty acids, and G the glycerol we have:

$$N \xrightarrow{\text{lipase}} F + G$$

Berczeller (1912) found lipolytic activity in all organs except blood and muscle. The spleen showed great lipolytic activity.

Volhard (see page 37) has shown lipolytic activity in the stomach.

Some have pointed out lipolytic activity in intestinal juice, while a great number have shown it to be present in the pancreatic juice or watery extracts of the pancreas.

The liver has been shown to be very active lipolytically (Leathes 1910), and a few investigators have stated that the blood possesses lipolytic activities. (Winternitz and Malay, 1910). The kidneys have been shown to be rich in lipase (Roeper, 1907).

Hona and Michaelis (1911) were able to show a lipase for mono- and tri-butyrin. It is particularly active in the serum and blood of rabbits and guinea pigs. It occurs less active in the blood serum of the dog, sheep, pig, and cow, the activity of the blood being as a rule greater than that of the serum.

Connstein (1904) argues against a lipase in the blood. Hanriot (quoted by Connstein) found that upon mixing serum with mono-butyrin there were produced butyric acid and glycerol. When the serum was heated to 65° C the splitting power was lost. At about 50° C the action was at its maximum. The antiseptics as chloroform, sodium fluoride, and a few others did not destroy the reaction. The splitting agent was not dialyzable. The agent is precipitated by alcohol and may be dissolved out from the alcohol by water. From these facts Hanriot arrived at the conclusion that there was in the blood an enzyme able to split fat, and he termed it lipase.

Connstein, on the other hand, came to the conclusion that Hanriot's lipase is nothing but an imaginary substance. He claims that mono-butyrin is so easily split that even water, acids, and especially alkalis will split it in comparatively large quantities. He further argues that many albumins are able to split the lower fatty acids. Finally he quotes Arthus and says that there may be in the blood a mono-butyrase but surely there is no such a thing as lipase. That this conclusion is erroneous will be seen later.

Ernst Freundenberg (1912) showed that the asceptically autolyzing liver is able to saponify fats to a marked degree. The latter in amounts up to 2 per cent of the liver substance are

hydrolyzed completely. The lipolytic power is destroyed by heat. The other organs are also lipolytic but in less degree. In order of lipolytic activity they rank as follows: spleen, muscles, lungs, kidneys, lymph glands, fatty tissue, and blood.

Loevenhart (1902) proved that the following organs contained lipase: pancreas, kidneys, liver, brain, adrenals, spleen, heart muscle, blood, and bile (the latter in the merest trace).

Adipose tissue (sub-cutaneous) was found very active, and it also had great synthetic power. Pericardial and perinephric fat were both found to be active, but markedly less so than the sub-cutaneous.

Kastle and Loevenhart (1900-1905) noted the activity of extracts of various organs of the pig. The following table shows the remarkable lipolytic activity of the liver. Taking the extract of the pancreas as unity, the lipolytic activity of the other organs stands in the following ratio:

Pancreas	1.00
Liver	3.93
Kidney	0.50
Submaxillary gland	0.36

Comparison of livers of different animals.

Table XXX

	% of hydrolysis
Pig liver	8.66
Sheep "	4.77
Duck "	3.70
Beef "	3.30
Chicken "	1.95

They also found a lipase in the extracts of the mucosa of the pig's stomach; however, it can not effect hydrolysis of fats in the presence of 0.3 per cent hydrochloric acid, which would indicate that any hydrolysis in the stomach would probably be confined to the first stages of gastric digestion.

Kastle and Loevenhart have further shown that the higher the molecular weight of the acid the more readily is its ethyl ester hydrolyzed by lipase, as can be seen from the following:

Table XXXI

	% of hydrolysis
Ethyl acetate	1.75
" proprionate	3.87
" butyrate	4.37

Kastle and Loevenhart (1900) showed that pancreatic lipase can form ethyl butyrate from butyric acid and ethyl alcohol. If the latter be true, as it is reasonable to believe, it throws a great deal of light on fat absorption.

Terraine (1909) showed that the optimum temperature of the pancreatic lipase is about 40° C, that it is quite active at 0° C, and ceases to be active at 54° C. Whether or not the different enzymes are identical is still a question, According to Kastle and Loevenhart, the enzymes of the liver seem to be identical, and the ester splitting enzyme of the liver differs from that of the pancreas.

Part V

Bacterial Action

We have discussed the most important factors in the absorption of fat, and it woudd not be amiss to mention in a paragraph or two the effect of bacterial action of fat absorption.

Very little has been investigated regarding bacterial action. Laxa (1901) noted that in the ether extract of butter there was an increase of the acid number from 2.7 to 47.7 after a month's standing. He could not detect glycerol and he concluded that it was used up as food.

Schreiber (1901) mentions several fat splitting organisms, e.g., spirilum Finkler, micro-coccus tetrogenes. Summaruga (1894) extended the list greatly. The question at issue is, are these organisms of any importance in the absorption of fat? Many authors give negative answers. Essereich (Connstein 1904) found many fat splitting organisms in the intestine of a suckling, but would not give weight to bacterial action in fat absorption. He found the following organisms:

Bacillus Subtilis--ability to split milk fat--36.19 %
" Lactis aerogenes " " " " " 34.34 %
" Coli " " " " " 62.70 %

Harley (1899) found that the removal of the large intestine in dogs did not increase the fat, fatty acids, or soap content of feces. This would indicate that the conclusion of Essereich (see above) is correct, since the large intestine undoubtedly contains the greatest number of bacteria.

Part VI

Resorption of Fat

There is no question at present that the animal fat is the main source of energy. Fat having an energy value which is about two and one-half times as great as that of either protein or carbohydrates (Rubner, Armsby, Zuntz and Wolf, quoted by Armsby) is most economically and conveniently carried in the body as reserve for the time of need and want. Its economy manifests itself in the small amount of energy necessary to maintain it in the body. A great many experiments have been conducted to show that the fat is the reserve energy made use of in starvation , excepting the fat of a few of the active organs which use fat at the expense of the adipose tissue, and consequently their fat percentage may not decrease.

The fats in the tissue serve as depots in an insoluble form. In cases of starvation they must be converted into a soluble form. A study of how the fat reserves are able to supply fat to other parts of the body in a soluble form is the purpose of these paragraphs.

Much experimenting as to fat resorption was done at the Experiment Station of the University of Missouri under the direction of P. F. Trowbridge (data unpublished).

Steers under different conditions of nutrition were studied and their growth noted. I shall give only a few data regarding the resorption of fat and its replacement by water.

Table XXXII

Growth of Steers under Different Conditions of Nutrition

Special Maintenance Animals

Offal Fat.

	#594 Check Steer	#597 Main-te-nance 6 mo.	#595 Main-te-nance 12 mo.	#591 Sub-Main-te-nance. 6 mo.	#592 Sub-Main-te-nance. 11 mo.	#593 Super-Main-te-nance 6 mo.
Wt. O. F. at Beg.	7.419	9.069	7.401	6.996	8.247	8.664
" " " " End	7.419	7.272	3.983	2.055	1.191	8.298
Gain in Offal Fat	--	-1.797	-3.418	-4.941	-7.056	-0.366
Wt. H_2O in O.F.Beg.	0.813	0.994	0.811	0.767	0.904	0.949
" " " " " "	0.813	1.307	1.249	0.799	0.972	1.048
Gain in H_2O in O.F.	--	0.313	0.438	0.032	0.068	0.099
% H_2O in Offal Fat	10.957	17.973	31.358	38.881	81.600	12.631
Wt. Fat in O.F.Beg.	6.371	7.788	6.355	6.908	7.082	7.440
" " " " " End	6.371	5.663	2.479	1.081	0.060	6.971
Gain in Fat in O.F.	--	-2.125	-3.876	-4.927	-7.022	-0.469
% Fat in Offal Fat	85.872	77.878	62.233	52.592	5.027	84.003

Table XXXIII

Growth of Steers under Different Conditions of Nutrition

Special Maintenance Animals.

Kidney Fat

	#594 Check Steer	#597 Main-te-nance 6 mo.	#595 Main-te-nance 12 mo.	#591 Sub-Main-te-nance 6 mo.	#592 Sub-Main-te-nance 11 mo.	#593 Super-Main-te-nance 6 mo.
Wt. K.F. at Beg.	1.934	2.361	1.927	1.821	2.147	2.256
" " " " End	1.934	2.392	0.596	0.220	0.176	3.424
Gain in Kidney Fat	---	0.031	-1.331	-1.601	-1.971	1.168
Wt. H_2O in K.F.Beg.	0.106	0.129	0.106	0.100	0.118	0.124
" " " " " End	0.106	0.179	0.160	0.043	0.143	0.166
Gain in H_2O in K.F	---	0.050	0.054	-0.057	0.025	0.042
% H_2O in K. F.	5.481	7.497	26.884	19.700	81.420	4.842
Wt. Fat in K.F.Beg.	1.802	2.199	1.795	1.696	2.000	2.102
" " " " " "	1.802	2.158	0.415	0.167	0.008	3.210
Gain in Fat in K.F	----	-0.041	-1.380	-1.529	-1.992	1.108
% Fat in K.F.	93.155	90.219	69.664	75.847	4.590	93.751

Table XXXIV

Growth of Steers under Different Conditions of Nutrition

Special Maintenance Animals

	#594 Check Steer	#597 Main-te-nance 6 mo.	#595 Main-te-nance 12 mo.	#591 Sub-Main-te-nance 6 mo.	#592 Sub-Main-te-nance 11 mo.	#593 Super-Main-te-nance 6 mo.
Fat in Lean Flesh						
Wt. Fat in L.F. Beg.	8.9	10.9	8.9	8.4	9.9	10.4
" " " " " End	8.9	8.7	5.4	2.9	1.6	10.8
Gain Fat in L. F.	---	-2.2	-3.5	-5.5	-8.3	0.4
% Fat in Lean Flesh	8.0	6.9	4.6	3.4	2.1	7.9
Fat in Skeleton						
Wt. Fat in Skel.Beg.	5.774	7.053	5.756	5.440	6.413	6.738
" " " " End	5.774	10.029	7.400	6.998	1.441	8.862
Gain Fat in Skeleton	---	2.976	1.644	1.558	-4.972	2.124
% Fat in Skel. at End	14.71	19.07	16.48	17.32	2.90	17.34
Fat in Soft Parts						
Wt.Fat in S. P. Beg.	38.35	37.87	30.90	29.21	34.43	35.98
" " " " " End	38.35	42.71	16.45	9.54	2.59	52.58
Gain Fat in S. P.	---	4.84	-14.45	-19.67	-31.84	-16.60
% Fat in S. P.	15.50	17.83	8.69	6.78	1.97	30.56

Table XXXV

Growth of Steers under Different Conditions of Nutrition

Special Maintenance Animals

Adipose Tissue.

	#594 Check Steer	#597 Main-te-nance 6 mo.	#595 Main-te-nance 12 mo.	#591 Sub-Main-te-nance 6 mo.	#592 Sub-Main-te-nance 11 mo.	#593 Super-Main-te-nance 6 mo.
Wt. A. T. at Beg.	25.63	31.31	25.55	24.15	28.47	29.91
" " " " End.	25.63	35.05	11.02	8.96	0.18	39.95
Gain in A. T.	----	3.74	-14.53	-15.19	-28.29	10.04
% Adipose Tissue End	10.36	11.59	4.47	3.84	0.06	13.83
Wt. H_2O in A. T. Beg.	4.88	5.96	4.87	4.60	5.42	5.70
" " " " " End	4.88	8.39	3.99	3.58	0.14	7.39
Gain H_2O in A. T.	---	2.43	-0.88	-1.02	-5.28	1.69
% H_2O in A. T. End	19.04	23.93	36.18	39.99	81.25	18.50
Wt. Fat in A.T. Beg	19.16	23.40	19.10	18.05	21.28	22.36
" " " " " End	19.16	23.43	5.59	3.91	0.01	29.30
Gain Fat in A. T.	---	0.03	-13.51	-14.14	-21.27	6.94
% Fat in A. T. End	74.75	66.86	50.75	43.64	4.55	73.36

Table XXXVI

Growth of Steers Under Different Conditions of Nutrition.

Composition of Special Maintenance Animals
Compared with Check Animal No. 594.

Entire Animal.

	#594 Check Steer	#597 Main-te-nance 6 mo.	#595 Main-te-nance 12 mo.	#591 Sub-Main-te-nance 6 mo.	#592 Sub-Main-te-nance 11 mo.	#593 Super-Main-te-nance 6 mo.
Moisture (Kilos)						
Wt. at beg.	138.2	174.5	142.4	134.6	158.7	166.7
Wt. at end	138.2	167.2	149.3	114.1	125.0	172.9
Gain	---	-7.3	6.9	-20.5	-33.7	6.2
% at end	57.7	57.2	63.7	63.0	69.2	56.3
Fat						
Wt. at beg.	44.1	55.7	45.5	43;0	50.7	53.2
Wt. at end	44.1	52.7	33.9	16.5	4.0	61.4
Gain	----	-3.0	-21.6	-26.5	-46.7	8.2
% at end	18.4	18.1	10.2	9.1	2.2	20.0

Only a glance at the above tables shows plainly how the sub-maintenance and even the maintenance animals use their fat reserves to keep them going. Steer No. 595 on maintenance for 13 months lost 61 per cent of its offal fat, 76.9 per cent of its kidney fat, and 65.9 per cent of its adipose fat. Submaintenance steer No. 591, losing about one-half pound per day, lost 82.1 per cent of its offal fat, 90.8 per cent of its kidney fat, and 79.6 per cent of its adipose fat. Neither of these have used any of their skeletal fat. Steer No. 592, sub-maintenance for 11 months, losing about one-half pound per day, lost 99.16 per cent of its offal fat, 99.6 per cent of its kidney fat, 99.92 per cent of its adipose fat and 77.5 per cent of its skeletal fat. In other words, he lost nearly all of his offal, kidney, and adipose fat, and more than three-fourths of the fat from the skeleton.

Rubner (quoted by Voit 1901) who experimented on a guinea pig which was not given any food for 8 days found that the animal had 9 per cent of its original calculated fat left.

Schimansky (quoted by Voit) found that a chicken after fasting 14 days had only 12 per cent of its original calculated fat left.

Falk (quoted by Voit) states that the dog on [illegible] the 28th day of fasting had only 10 per cent of its original fat left.

Comparing the work of the different investigators we see that there is a relatively fair agreement.

Table XXXVII

	% Fat left from originally calculated
Trowbridge --Steer 592 (sub-maintenance 11 mo. losing ½ lb. per day)	7.9
Rubner ------Guinea pig (starved 8 days)	9.0
Falk --------Dog (starved 28 days)	10.0
Schimansky---Chicken (starved 14 days)	12.0

Liver

The percentage of fat in the liver does not seem to decrease during starvation. Whether this is due to a constant building up of fat or to a conveying of fat to it from the adipose tissue is not known.

Patton (1895) experimenting on kittens, obtained the following results:

Four young kittens of a litter produced on June 20th. They had suckled in the morning.

A weighed 214.5 grams
B " 204.5 "
C " 214.0 "
D " 199.0 "

A and B were killed at 4 P. M., about 8 hours after receiving food. Milk was present in the stomach of both.

C was kept without food till 5 P. M. on the 21st, 33 hours, and then killed. It weighed 203 grams. The stomach was empty. D. was kept without food till 3:30 P.M. on the 22d, 56½ hours, and then killed. It weighed 175 grams and the stomach was empty.

Table XXXVIII

Liver

	Wt. of Liver	Ether extract		Fatty acids		Length of fast
		% of liver	% of solids	% of liver	% of ether extract	
A	9.0	4.15	15.0	2.63	55.9	8 hr.
B	7.5	4.50	15.8			
C	6.0	*7.36	26.6	2.53	45.3	33 hr.
D	5.5	4.05	19.5			56½ "

*Ether extract imperfectly dried.

Twelve pigeons were procured on September 20th and fed on bruised oats and water for four days.

A and B were killed September 25th
C and D " starved till Sept. 26th, then killed
E and F " " " " 27th " "
G and H " " " " 29th " "
J and K " " " " 30th " "

Table XXXIX

	Wt. of liver	% of solids less ether extract	Ether extract		Period of fast hrs.
			% of liver	% of solids	
A	5.2	22.8	4.37	16.06	0
B	6.7	21.0	4.46	17.04	
C	4.2	22.7	3.92	14.7	24
D	3.5	24.1	4.31	15.1	
E	6.2	18.0	3.25	11.1	48
F	4.2	24.6	3.73	10.3	
G	4.2	23.2	5.40	18.8	72
H	4.5	22.4	4.46	16.6	
J	2.8	24.1	4.68	16.2	96
K	4.3	25.8	5.04	16.3	

Table XL

Steers (under investigation at the Expt. Station of the University of Missouri.)

Comparison of the per cent of ether extract of the livers.

Age months		% ether extract
12	594-Check animal	5.365
18	597-Maintenance 6 mo.	2.698
23	595-Maintenance 12 mo.	3.360
18	591-Sub-maintenance 6 mo.	2.174
22	592-Sub-maintenance 11 mo.	3.739
18	593-Super-maintenance 6 mo.	2.223

From Patton's and Trowbridge's results, the conclusion would be that the fat in the liver of starving animals is not decreased.

Blood

Schultz (1896) made the assertion that during starvation the fat content of blood increased. In several cases the increase was 100 per cent. Schultz digested the blood with pepsin-hydrochloric 0.1 per cent solution at 60 to 75° C for 24 hours. He dried the blood and extracted it with ether--He calls this fat, altho admitting that it contains several other things besides fat.

Lattes (1908) working with the Kumagawa-Suto method found the fat content of the blood of the normal dog to be 0.3 to 0.42 per cent, and that the venous blood seemed to be higher

in fat than the arterial blood. After a fatty meal he sometimes found the fat content increased about 100 per cent. He did not obtain a fat increase in the blood during the starvation period. This differs from the conclusion arrived at by Schultz (see page 90) under similar conditions.

Freundenberg (1912) confirmed Latte's work and found the petroleum ether extract of blood always constant.

Table XLI

Dogs

	Normal Petroleum ether extract	During Starvation	Days of Starvation
Fat (Pomeranian dog)	0.877	1.008	4
Lean (Fox terrier)	0.537	0.531	4

Rabbits

Wt. of rabbit gms.	Normal Petroleum ether extract %	During Starvation	Days of Starvation	Reduction in body weight
3100	0.356	0.328	5	16
3240	0.331	0.292	5	15
3100	0.372	0.380	7	30

If blood be withdrawn and analyzed for fat and in 24 hours another sample of blood be withdrawn and analyzed, the results are almost identical, showing that there exists a kind of equilibrium.

Table XLII

Rabbits

Wt. of rabbit grams	Blood withdrawn grams	Fat %	After 24 hours
1600	36.8	0.384	0.338
1700	26.3	0.247	0.299
2100	29.7	0.376	0.264

This constancy of fat in blood confirms the work of many investigators that an animal lives on its own fat, and therefore there is always a fat mobilization from the reserve fat to the different organs of the body.

Part VII

Experimental

As I shall point out later in my discussion, many of the results of the experiments conducted by investigators are by no means very reliable, due to the fact that the methods used will give more or less inaccurate results. There are several methods for fat determination. They are: the Soxhlet method, the Rosenfeld method, the Dormeyer method, the Glikin method, the Liberman and Szikely method, and the more modern Kumagawa-Suto method.

The Soxhlet method , in principle, is really a determination of the ether soluble material in the substance under investigation. It has been shown that besides fat it will dissolve out the cholesterols, lecithins, and other phosphotide and nitrogen containing compounds (Shimidzu, 1910).

The Rosenfeld method consists in placing the powdered material in a Soxhlet tube suspended in a beaker containing absolute alcohol, and boiling on the water bath for one-half hour. In order to prevent loss of alcohol a round flask through which cold water circulates is placed on the beaker. The tube is next placed in a Soxhlet extractor and extracted with chloroform for 5 hours. The whole process is then repeated. The combined filtrates are then extracted with ether and dried and weighed.

By the Dormeyer method the powdered substance is digested with 100 c.c. of 0.5 per cent hydrochloric acid and 0.2 per cent of Grübler pepsin, at 40° C for 120 hours. After drying, the material is extracted with ether in a Soxhlet apparatus. The ether is evaporated and the residue again treated with ether.

The filtrate and wash water are extracted for 24 hours with ether in a fluid extractor, evaporated, and again treated with ether.

Glikin's method consists in drying the powdered substance under a vacuum at 60-65° C for several days. The cones are next dried in an oven for two days. The powder thus treated is extracted with petroleum ether for 72 hours.

The Liberman and Szikely method is in principle the same as the Kumagawa-Suto. The difference is that Lieberman and Szikely used an aliquot of their petroleum ether and titrated it with alcoholic KOH and from the dried soaps they calculated the amounts of higher and lower fatty acids together as the glycerides. The general method of manipulation is different. Liberman and Szikely use a long necked flask, which up to the middle of the neck contains 290 c.c., the neck having a diameter of 3.6 c.m. Five grams of substance is boiled with 30 c.c. of a 50 per cent KOH solution for one-half hour, on an asbestos wire gauze. After cooling 30 c.c. alcohol is added and again warmed. Twenty c.c.of a 20 per cent sulfuric acid solution is next added. After cooling it is treated with 50 c.c. petroleum ether, stoppered and shaken thirty times, 10 seconds each time. The flask is then filled to the mark with a saturated sodium chloride solution, and again shaken several times. From the well separated petroleum ether layer 20 c.c. is pipetted off and treated with 40 c.c. ethyl alcohol. Phenolphthalein is added (1 c.c. of a 1 per cent alcoholic solution = 0.01 gm), and titrated with an alcoholic KOH solution. They use the following formula for calculating results:

$$F = \frac{S - 0.01 - (K \cdot 00255)}{A} \quad .250$$

Where F = fat; S = amount of soaps; K = amount of N/10 KOH; and A = amount used.

Kumagawa and Suto (1908) have made a thorough study of all the above methods, and have shown conclusively that none of them give accurate results, with the possible exception of the Lieberman-Szikely method. As is to be noted, however, the latter method is very complicated and if not followed literally will give different results in the hands of different workers.

Kumagawa-Suto method: The fresh sample containing fats is placed in a beaker and boiled on the water bath for 2 or 3 hours with 25 c.c. of a 5N NaOH solution (20 gm. in 100 c.c. of solution). Boiling is best accomplished by placing the beaker in the water bath and covering it with a funnel. During this saponification the mixture is stirred a few times. The solution while still hot is placed in a glass stoppered separatory funnel of about 250 c.c. capacity. The beaker is washed out two or three times with a little warm water (5 c.c.). The solution is then over-neutralized with 30 c.c. of a 20 per cent hydrochloric acid solution (sp. gr. 1.1). (Before addition of the acid the solution is cooled to about 50° C, and 20 c.c. of the HCl added and well shaken; the other 10 c.c. is next added and treated as before).

After thorough cooling 80 c.c. of $(C_2H_5)_2O$ is added and vigorously shaken. A precipitate separates itself in a distinct layer between the water and ether layers. The clear watery solution is poured off. The brownly colored ether is carefully placed in a beaker.

The funnel with the precipitate is washed twice with a small amount of ether (5-10 c.c.). The precipitate is again dissolved by shaking in 5 c.c. NaOH. The watery solution is now placed in the funnel and again well shaken. The reaction is now acid and the remaining fatty acids are taken up quantitatively by the ether. The combined ether solutions are evaporated, again taken up with ether, and filtered through asbestos. This filtrate contains besides fatty acids, coloring matter, lactic acid, and other unsaponifiable materials. It is thoroughly dried at 50° C (on the water bath for about 3 hours) and taken up with petroleum ether. For this purpose 25 c.c. of petroleum ether is added gradually while the dried ether extract is still hot. The beaker is covered with a watch glass and permitted to stand from one-half to one hour. The petroleum ether is next filtered thru asbestos. The filtrate (which is supposed to be colorless, but which I was never able to obtain) is evaporated on the water bath and dried to constant weight, which takes only a short time.

Quantitative Separation of the Unsaponifiable Substances (Including Cholesterol from the fatty acids.

The above residue is treated in a separatory funnel with 30 c.c. petroleum ether. To this is added four to five times its volume of .2N absolute alcoholic potash or soda. It is shaken vigorously. As much water is now added as the alcoholic alkali. The alcoholic concentration is now 50 per cent (by volume). A separation occurs here, the unsaponifiable substances remaining in the petroleum ether while the alcoholic solution takes up the soaps. The alcoholic solution is again shaken with 30 c.c. of petroleum ether. The combined ether solution is evaporated and

the residue again taken up with 10 c.c. of alcohol, treated with 1 c,c. of .1N alcoholic NaOH, evaporated on the water bath and dried at 100° C from 15 to 30 minutes. The residue while still hot is extracted with petroleum ether, filtered thru asbestos, evaporated and dried at 100° C to constant weight.

Several determinations of fat in blood were run by the **Shimidzu** modification of the Kumagawa-Suto method. Twenty grams of blood was treated with four times its volume of cold alcohol (95%) and after **2 or** 3 hours filtered thru a rag, and the alcohol pressed out. The residue was then extracted in a soxhlet with boiling alcohol. The combined filtrates were boiled on the water bath from 1½ to 2 hours (in a beaker) with 25 c.c. of 5N NaOH, and continued as in the Kumagawa-Suto method (page 95).

Table XLIII

Blood

Sample	Wt. of sample gms.	Wt. fatty acids & unsaponifiable substances gms.	Wt. of unsaponifiable substances gms.	Wt. of fatty acids gms.	Wt. of neutral fat gms.	% of neutral fat
Cow blood	20	0.1755	0.0813	0.0942	0.09853	0.4927
"	"	0.1785	0.0935	0.0850	0.08891	0.4446
"	"	0.1523	0.0967	0.0556	0.05816	0.2908
"	10	0.0782	0.0471	0.0311	0.03253	0.3253
"	9.5	0.0757	0.0353	0.0404	0.04226	0.4444

Average 5 determination 0.3999 per cent

Greatest variation from average 28 per cent

Least " " " 11 " "

As can be seen from the above table the results are far from

satisfactory. The great difference in the results may be due to several causes. First, the great number of filterings of the ether and petholeum ether would undoubtedly cause a great loss. Second, and most probably a very important error is the great amount of cholesterol and other unsaponifiable substances, which amount to over 50 per cent of the total, soluble in petroleum ether. This may therefore cause difficulty in separating it from the fatty acids. Another probable error is the impossibility of getting rid of all the coloring matter after thorough drying of the ether solution (see method) It was found that the longer theether solution is dried on the water bath, the less percentage of fat in blood is obtained. Nos. 1 and 2 I dried on the water bath for three-fourths hour. I obtained 0.4927 and 0.4446 per cent respectively. No. 3 I dried on the water bath for 6 hours. It gave a larger precipitate on adding the petroleum ether. I obtained 0.3908 per cent. Even after 6 hours drying I was unable to obtain a colorless filtrate. It was colored light yellow. Nos. 4 and 5 were dried on water bath for 3 and 4 hours respectively They did not seem to give as large precipitates as did No. 3. The percentages were 0.3253 and 0.4444 respectively. It seems to me that this plainly shows that the longer they are dried the less percentage of fatty acids will be obtained. Whether this is due to oxidation or to the driving off of the last amounts of ether, as is suggested by Kumagawa-Suto, has not been determined. It seems that the former may be the more reasonable since the ether which boils at 38° C would be driven off on the water bath when the temperature was between 75° and 95° C.

Another probable source of error is the long drying on the water bath, which may cause some of the fatty acids to become insoluble in petroleum ether (Hartley, 1909, and substantiated by Bloor,*1914).

In order to determine whether the great amount of cholesterol and other unsaponifiable substances in the blood soluble in petroleum ether have really a great deal to do with the fluctuation of results we determined the amount of fat in the liver of the same cow whence the blood was obtained, since it is stated by Patton (1895) that the liver contains only about 0.03 to 0.05 per cent of cholesterol.

One hundred grams of liver were saponified and made up to volume , 1000 c.c., with water; 100 c.c. aliquots representing 10 grams of sample were then taken and analyzed.

Table XLIV
Liver

Sample	Wt.of sample gms.	Wt.fatty acids & unsaponifiable substances gms.	Wt. of unsaponifiable substances gms.	Wt. of fatty acids gms.	Wt. of neutral fat** gms.	% of neutral fat
Cow liver	10	0.2125	0.0194	0.1931	0.20198	2.0198
"	"	0.2305	0.0273	0.1934	0.20230	2.0230

*It should be mentioned here that Bloor very recently published a new method, called the Nephelometric method, for small amounts of fat in blood.

**The factor 1.046 was used to obtain the weight of neutral fat from fatty acids (Kumagawa-Suto).

It is interesting to note how closely these two results agree, within 0.003 of a per cent. It is more interesting because of the fact that the weights of the two fatty acids plus the cholesterols and other unsaponifiable substances differ as much as eight milligrams, but the weight of the cholesterols differs in about the same amount and makes up for the results. This same fact may be noted from Shimidzu's tables (1910). This last table also shows that the amount of unsaponifiable substances in the liver soluble in petroleum ether is over 10 per cent of the total amount. This close agreement, it seems to me, would indicate that the less the amount of cholesterol the less the errors introduced, regardless of the other errors enumerated above.

I next determined the fat in the back fat of a hog. Here I found difficulty in saponifying the fat of which I took about 6 grams. After boiling it on the water bath for 5 hours with 30 per cent sodium hydroxide there was still a heavy layer of fat on top. I finally discarded the same, weighed out a sample of the same material (3.8363 grams) and added 50 c.c. of a 30 per cent sodium hydroxide solution and 50 clc. of 95 per cent alcohol, and saponified it for 3 hours under a reflux condenser. There was thorough saponification. The alcohol was then boiled off as completely as possible, and made up to a liter of solution with water and 100 c.c. aliquots taken. The petroleum ether was perfectly colorless.

Table XLV

Back Fat of Hog

Wt. of sample gms.	Wt. fatty acids & unsaponifiable substances gms.	Wt. of unsaponifiable substances gms.	Wt. of fatty acids gms.	Wt. of neutral fat gms.	% of neutral fat
2.8262	0.2404	0.0010	0.2394	0.2504124	88.62
"	0.2356	0.0009	0.2347	0.2454962	86.87

It is to be noted here that the agreement is not as good as might be expected, most probably due to the several sources of error in the method.

Soxhlet method: No ether soluble material was obtained in the blood. The percentage of the ether soluble material in the liver amounted to 2.722.

I analyzed the fat of loin of hog according to the Soxhlet method and the following table gives the results:

Table XLVI

Wt. of sample gms.	Loss in wt. due to drying gms.	% of moisture	Wt. of dried cones + fat gms.	Wt. of cone after extraction gms.	Loss in weight gms.	% of fat in ether soluble
0.9880	0.0759	7.68	2.7932	1.9027	0.8910	90.13
1.8565	0.0769	6.13	3.0347	1.8845	1.1502	91.64

I have every reason to believe that number 1 is the right one, that is, the percentage of ether extraction is 90.13, because the percentage of moisture in the second is 1.55 per cent

lower than it really should be. If we subtract that 1.55 per cent from 2, we get 90.09 per cent or an average of 90.11 per cent. I shall use this average in my further discussion.

As can be seen from the results the Kumagawa-Suto and the Soxhlet methods by no means agree. Especially is this true with blood and liver fat. The former method undoubtedly gives the more accurate results, but the difficulty is with the great number of errors introduced by the amount of cholesterol and other unsaponifiable substances and by too many filtrations; and also probably by too much technique.

In order to work out a quick and at the same time an accurate method for fat determination, one that could be made use of in a laboratory where a great many fat determinations are run, different modifications of the Kumagawa-Suto method were tried. I shall not go into a detailed account of the modifications tried, for it would take too much space, and would be of little value. I shall propose only one modification which we think very accurate and at the same time quick.

The sample, if it does not contain much fat, is directly saponified with 5N NaOH, etc., according to the Kumagawa-Suto method as described above. The combined etherial solutions are evaporated to dryness and let stand on water bath from 1 to 2 hours. It is taken up with about 25 c.c. petroleum ether and about 10 or 15 c.c. of 95 per cent alcohol added. This is titrated with N/20 alkali, using about two drops of a 1 per cent solution of phenolphthalein as indicator. The end point is sharp and distinct.

With a sample of blood an alcohol extraction should first be made as recommended by Shimidzu. With a sample rich in fat I should recommend that saponification be brought about in the presence of alcohol, using a reflux condenser to prevent any loss in the alcohol; after saponification the alcohol can be driven off by boiling.

The following tables give the analyses of blood, liver, and back fat of a hog by the modified modification.

Table XLVII

Blood

Wt. of sample gms.	Number of c.c. of 0.049926/N NaOH	Wt. of NaOH gms.	Wt. of fatty acids gms.	Wt. of neutral fat gms.	% of fat
20	3.6	0.0071893	0.04976	0.05205	0.2602
"	3.5	0.0069896	0.04840	0.05063	0.2531
10	1.85	0.0036945	0.02558	0.02676	0.2676
9.5	1.78	0.0035547	0.02462	0.02575	0.2716

Average per cent = 0.2628

Greatest variation from average = 3.7 per cent

Least " " " = 0.95 " "

This fat determination shows very concordant results, and although it differs greatly from the average of five determinations of the same sample by the Kumagawa-Suto method, one surely would be more apt to take these latter results than the former, whose greatest variation is 28 per cent, while the greatest variation by the proposed modification is only 3.7 per cent.

Table XLVIII

Liver

Wt. of sample gms.	Number of c.c. of 0.049926/N NaOH	Wt. of NaOH gms.	Wt. of fatty acids gms.	Wt. of neutral fat gms.	% of fat
10	13.45	0.02648	0.1834	o.19183	1.9183
"	13.50	0.02696	0.1842	0.19269	1.9269
"	14.50	0.02896	0.20052	0.20974	2.0974

Average per cent = 1.9819

This table again shows how concordantly the results run.

Table XLIX

Back Fat

0.282682	17.62	0.035188	0.243676	0.2548851	90.18
"	17.60	0.0351479	0.243399	0.2545954	90.08

Average per cent = 90.13

In all the above experiments a large sample was taken and aliquots used.

Table L

Comparison of Three Methods

Substance	Method	% of fat
Blood	Soxhlet	0.0
"	Kumagawa-Suto	0.3999
"	Modified	0.2628
Liver	Soxhlet	2.7220
"	Kumagawa-Suto	2.0214
"	Modified	1.9818
Fat of loin of hog	Soxhlet	90.11
" " " " "	Kumagawa-Suto	87.74
" " " " "	Modified	90.13

As can be seen from table XLIV, the Soxhlet method does not compare at all favorably except when working with almost pure fat. With the blood the ether did not extract any thing, while with the liver it extracted 35 per cent.of substances other than fat. This agrees with Patton (1895) who claimed that lecithin is a constant constituent of ether extract of the liver. The Kumagawa-Suto method has many errors as has been pointed out previously and is quite time consuming. The modified method, on the other hand, is very short and by no means complicated. The modified method,besides being economical in time, about 75 per cent, is very economical in materials; there is no waste of petroleum ether as in the Kumagawa-Suto method, for the petroleum ether and alcohol used in titration can easily be separated in a funnel and re-distilled. The method contains one weak point, however, and that is that in titrating a molecular weight has to be assumed. Kumagawa and Suto in working on fats of different animal organs found that in the main they consist of 70 per cent oleic acid, 20 per cent palmitic acid, and 10 per cent stearic acid. If this be true, then one would multiply the molecular weight of oleic acid by 7, palmitic by 2, and stearic by 1, and divide the results by 10. I did that and obtained a molecular weight of 277, very close to the molecular weights of oleic and stearic acids. That 277 was a fair estimate of the mean molecular weight can be seen from the fair agreement in the results, except in the blood determination. It would seem at first glance that if the Kumagawa-Suto method in blood is correct, but

on examining it a little closer one finds that even if one assumes the fat to consist all of oleic acid, which has the highest molecular weight of all then the results do not differ materially , as can be seen from the following table.

Table LI

Computing the amount of neutral fat from the determination of Table XLVII, using the molecular weight of oleic acid (282).

Wt. of Sample gms.	c.c. of 0.049926N NaOH	Wt. of NaOH	Wt. of Fatty Acids gms.	Wt. of Neutral Fat gms.	% of Neutral Fat
20	3.6	0.0071893	0.051845	0.051229	0.2711
20	3.5	0.0069896	0.049277	0.051483	0.2574
10	1.85	0.0036945	0.026046	0.027243	0.2724
9.5	1.78	0.0035547	0.025311	0.026475	0.2787
		Average	0.2699		

If we assume that there exists an equal amount of all the three acids the results do not differ either, as the average of the three equals 273. It is not necessary to go to the other extreme, that is, to take the molecular weight of palmitic acid (256) as the mean, for in no case did the proposed method give much higher results than the Kumagawa-Suto method.

Summary of Methods

I shall not go into a detailed accout of the methods used in various experiments by the different investigators. Many of the authors do not state what methods they used, and as I have

pointed out, most of the methods are more or less faulty and many of our conclusions may be more or less erroneous. The histological methods have been shown to give more or less inaccurate results. Noll (1910) concludes that fat must traverse the mucous membrane of the intestine not merely in the form of substances reacting with osmium, but also in some other form.

Kumagawa and Suto, who have studied nearly all the methods in detail, state that hardly any of the results obtained by the various methods agree. The proposed their method (which is really more or less a modification of the Lieberman-Szekely method) which is admitted by all recent workers to be the most accurate of all methods so far advanced. As demonstrated above, however, it contains many sources of error, and especially in substances containing much cholesterol, or other unsaponifiable substances soluble in petroleum ether. In the proposed modification most of the sources of error of the Kumagawa-Suto method are eliminated. It has the advantage of both economy in time, about two-thirds, and economy of material, there being no waste of petroleum ether. Finally, the method has the greatest of all advantages in the concordance of results.

It is to be hoped that with a method easily handled and one that will give reliable results, further work regarding fat absorption and resorption may be undertaken, so that our knowledge regarding these subjects will be greatly increased.

Part VIII

General Discussion of the Results of Investigations Concerning Fat Metabolism

Of the many able workers who helped to supplant the old view, Pflüger, Rochford, Kastle, Loevenhart, Volhardt, Fromme, Zinsser, and Wohlgemuth should be mentioned. We shall discuss the present views by considering briefly the process in the different organs.

Stomach

Most investigators agree that there is some splitting of neutral fat in the stomach. Among the most prominent men who doubt the existence of a lipolytic agent in the stomach are London and Wersilowa (1908), Connur and Niclaux (1910), Connstein (1904), and several others. But, on reviewing the literature, there remains no question in one's mind as to the capability of the stomach to split fat. Zinsser, Fromme, and Wohlgemuth have shown conclusively that the mucosa of the stomach and the gastric juice do possess great lipolytic power. That the lipase is derived from the stomach wall and not from regurgitated pancreatic juice has been shown by Ibrahim and Kopek on newly born children, and by Fromme (1903) on the mucosa of stomachs of dogs and hogs, showing that the lipase is found only in the fundic region of the stomach, and if the lipolytic action were due to regurgitated pancreatic juice it should be found more active in the latter region, which is not the case.

The presence of a fat splitting enzyme in the stomach may furthermore explain the fact that even after extirpation of the pancreas there may frequently occur absorption of fat, especially the naturally emulsified fat of milk.

That fat may also be absorbed in the stomach, and that it is not inconsiderable has been shown by Volhard (1900-1); Greene (1912-13); and Weiss (1912).

Intestine

The question whether or not there is a lipase in the intestinal juice apart from the lipase of the pancreatic juice is still undecided, although experiments seem to favor the affirmative. Lombroso (1908) claims that even if the pancreas be extirpated there is still a splitting of fat in the intestine. This is supported by Boldyreff's experiments (page 44) who showed that dogs whose pancreas were extirpated were able to digest milk fat. As I pointed out previously, however, this digestion and absorption of emulsified fats like milk fat, may be due to the splitting of the fat in the stomach, and therefore Boldyreff's conclusions may be incorrect. Furthermore, Heidenhuas (page 44) has pointed out that many albumins in the intestine are able to split fats.

Falk (1914) very recently has claimed to have found two enzymes in the duodenal juice. He does not put forth any theory whence they were derived. Loevenhart claimed that the pancreatic juice contains one lipase, and if that be true, we would be apt to believe that the other enzyme claimed by Falk is derived from the intestine.

Liver

The importance of the liver in fat metabolism aside from its bile producing properties, has been recognized only recently. The fats occurring in the liver are more unsaturated than those found in any other organ. The exact meaning of the presence of these unsaturated acids is not yet clear. Does the presence of these highly unsaturated acids indicate that they represent a stage in the normal breakdown of fat in the liver, or does it represent a stage in the synthesis of fats, or are the acids essential constituents of the protoplasm of the cell which enables it to carry out functions not directly associated with the katabolism of the acids themselves.

It has been argued that the fats found in the liver are due to the production of fats from carbohydrates (page 47) That this is undoubtedly true to some extent is not denied, but that all the fat in the liver should be produced from that source is a wrong conception. Rosenfeld's experiments (page 47) clearly point out that large amounts of fats in the liver are due to an importation of storage fat from the connective tissue. Lebedoff and Rosenfeld have shown conclusively that liver fat varies in character with the adipose fat. The liver, therefore, seems to be the organ to which the migration of fat from other parts of the body occurs. Here the fat either begins to be oxidized and is carried to the organs of the body to be finally oxidized and consumed, or it may be a special function of the liver to build up out of the connective tissue complex combinations of lecithin

and other phosphotide lipoids, not only for the use of its own cells, but it may be for the use of other organs as well. To sum up the fat metabolism of the liver, the knowledge of which is very meager, we must assume that the liver takes up the fat from the arterial blood, gets to work on same at once, and brings about those changes described above. When an animal is underfed the liver will take up the fat of the adipose tissue and distribute it to the different organs in proper condition. The liver probably accomplishes this by either of the two following methods, or by the two combined. First, by "desaturation" and second, by synthesizing the fatty acids into those which contain several double links.

Bile

It has been proved conclusively that the bile assists in the absorption of fat. How it does it, however, is a matter of much dispute.

Pflüger (1901) attributed its action to its solubility on soaps. Others think that it activates the pancreatic lipase (Howell, 1911), and still others believe that it contains a special lipase.

It has been pointed out by Loevenhart and Souder (1906) that the pancreatic juice without the bile would only split one-sixth of the fats that it normally does, or, in other words, the fats would pass through the duodenum with only one-sixth of the amount of splitting that normally occurs. They have also shown

that the bile has only the merest trace of lipolytic action, and therefore its great activity can surely not be due to a lipase.

How the bile activates the pancreatic lipase has been a question of much discussion. Hewlett (1905) pointed out that it is due to the lecithin in the bile, while Loevenhart and Souder (page 53) state that the effect is due to the bile salts. This latter view has many supporters, and seems to be the true conception. I should mention here that it is an undisputed fact that the bile, due to its sodium carbonate, has a great emulsifying power, which would give the lipase more surface to act upon, and consequently favor a greater splitting.

Pancreas

No one at present can dare deny the lipolytic power of the pancreas, and that this power is of the utmost importance in the absorption of fat. The failure of many investigators to obtain a diminished absorption of fat after ligating the pancreatic ducts was evidently due to the fact that pancreatic juice continued to enter the intestine.

Pancreatic juice, due to its lipolytic power, has indirectly an effect on emulsifying the fats due to the fatty acids liberated (page 57). The lipolytic action is rapid and complete. Loevenhart (1902) has shown that the pancreatic juice is capable of hydrolyzing all the fat of a fatty meal in the period of pancreatic digestion.

Harley and Lombroso (pages 57 & 58) have shown that if the pancreas is extirpated the amount of fat excreted is more than

the amount of fat fed. Lombroso points out that the pancreas has an internal function whose presence is necessary in order that the fat stored up in the tissues may be rendered available for the organism. Not much experimenting has been conducted to prove this point, and Lombroso's conclusions are still in doubt.

As I have stated above, no one at present denies the lipolytic activity of the pancreatic juice. Wohlgemuth (pp.70 & 72) has clearly shown that on a fatty meal the lipase concentration of the pancreatic juice is by far greater than after either protein or carbohydrates.

Absorption

There is a variance of opinions as to how the products of cleavage are absorbed. Some claim that they are absorbed as free fatty acids, while others believe that they are absorbed as soaps. The more general opinion is that the fats are absorbed as fatty acids; very little, if at all, as soaps. Moore and Rockwood (1897) concluded that fats are absorbed as fatty acids and soaps. They ask the question, why is it impossible to assume that a small amount of alkali should not act as a carrier to convey all the fat in the form of soap into the epithelial cells? They assume that soaps may be split in the epithelial cells, sending back the alkali into the intestine, where it can combine with a fresh amount of fatty acids, the same process being repeated till all the fat is absorbed. Kastle and Loevenhart (1900), however, were unable to synthesize ethyl butyrate from sodium butyrate and ethel alcohol by means of lipase, which would contradict Moore and Rockwood's assumption. The former's inability to syn-

thesize the ethyl butyrate from the sodium soap is in harmony with the facts which point to the non-existance of soaps in the body.

How fat is absorbed from the epithelial cells of the central lacteal is a question of much controversy. Some claim that fat is absorbed as such, while others claim that the fat is absorbed in solution and that it is hydrolized before absorption. One of the difficulties in finding out what products are poured into the blood is the fact that we have no method at present by means of which to determine how the fat exists in the blood. If the theory of many of the investigators be correct, that is, that there are lipases in the blood, then we must assume that the latter contains fatty acids. The question then is, why is the blood usually alkaline? The blood, furthermore, can not contain soaps since Munk has shown that soaps in the blood, when injected, are very poisonous. We can assume either of two propositions for the fat content of blood: first, that the blood contains neutral fat, and, second, that it contains soaps of cholesterol or of other unsaponifiable substances. This second assumption would again force to the front the question of the splitting of fat in the blood. Wells, and Abderhalden state that the fatty acid and glycerol are united in the blood to form neutral fat and are removed from the blood very soon after they are formed. This agrees well with the work of Kastle and Loevenhart and others who found that the blood has lipolytic activity, and furthermore, fits in well with the fact that the blood is alkaline.

Part IX

Summarized View of the Processes of Fat Metabolism

Absorption

The action on fats starts in the stomach. The lipase splits them into fatty acids and glycerol, and some absorption takes place. Upon reaching the intestine lipolysis again starts, principally by the action of pancreatic juice, assisted by the bile either as a solvent or emulsifying agent, or both, with the production of a mixture of fat, fatty acids and glycerol. The fat is separated from the fatty acids and glycerol by absorption of the latter into the walls of the intestine. Hence an equilibrium is not reached in the intestine, and so the splitting continues until practically all the fat has been decomposed and the products absorbed. When, therefore, the mixture of fatty acids and glycerol first enters the epithelial cells lining the intestine there is no equilibrium. The lipase immediately sets about to establish an equilibrium, combining them. As a result the cell mixture of fat, fatty acids, and glycerol, will attain equilibrium only after fatty acids and glycerol cease to enter the cell. Now another factor also enters, for on the other side of the cell is the tissue fluid containing relatively little fatty acid and glycerol. Into this the diffusible contents of the cell will tend to pass to establish the osmotic equilibrium which is quite independent of the chemical equilibrium (Wells, 1903). This obstruction of part of the cell contents tends to overthrow chemical equilibrium again, there now being an excess of fat in the cell. The lipase will,

under this condition, reverse its action (Kastle and Loevenhart, 1900) and split the fat it has just built into fatty acids and glycerol.

In the blood serum, and also in the lymphatic fluid, there is more lipase which will unite part of the fatty acids and glycerol, and by removing them from the fluid about the cells favors osmotic diffusion, which facilitates absorption. The rpocess in the tissue cells must be exactly as described above. According to this description, there is throughout the body constantly taking place both splitting and building up of fat. The latter is a resting stage, or in other words, it is an energy reservoir, mainly, besides the few other subordinate functions it may have.

Resorption

In the light of the above description we can easily see how fat is resorbed in case of starvation. Kastle and Loevenhart have shown that subcutaneous fat and the other fat reserves are very active lipolytically. In time of want and hunger, the animal makes use of its own fat by setting into action its lipase which will break up the neutral insoluble fat into fatty acids and glycerol. These, by osmotic diffusion, are carried by the circulatory system to the different organs of the body for consumption. It is probable that they are carried to the liver first and from this organ distributed to the other active organs of the body. It has been pointed out by Loevenhart that pericardial and perinephric fat were both found to be markedly less active than sub-cutaneous fat, and that during starvation they

are the last to disappear. This fact would support the above theory that fat must first be split before it can be resorbed, and that the fat of those organs having more lipolytic power would necessarily be the first to disappear.

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