chloride solution with a maximum solubility at the concentration 1.5 *N*. Duodenal contents contain an esterase and a lipase, the former predominating in the intestinal juice, the latter in the pancreatic juice and bile.

Marked similarities in the action of neutral salts and alcohols are shown by the lipases from different sources.

The action of heat and of drying on the soy bean lipase was found to be similar to their action on castor bean lipase and esterase.

The analyses of the soy bean lipase preparations showed no marked differences in comparison with the analyses of the castor bean preparations.

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[CONTRIBUTION FROM THE BIOCHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS.]

ENZYMES OF THE CENTRAL NERVOUS SYSTEM.

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The enzymes of the brain and nerves have not been investigated as thoroughly as those of most other tissues. This is rather surprising when one considers the supreme importance of the nervous system. Most of the previous data on brain enzymes has been obtained as part of other investigations, the enzymes of the nervous system having been considered only incidentally. The work here presented had for its object the beginning of a more systematic study of the enzymes of nervous tissue and a somewhat more detailed study of the most important ones.

Preparation of Extracts.

For the larger part of this work fresh sheep brains, packed in ice soon after removal from the animals and expressed directly from Chicago, were used. The membranes and blood vessels were removed and the brain divided into cerebrum, cerebellum, medulla, mid-brain, and, in some cases, corpus callosum. These divisions were minced separately in a meat grinder, then ground to a fine state in a mortar, with either toluol or oil of mustard as a preservative, usually the latter, and extracted with water 0.2% HCl, 0.9% NaCl, 0.5% Na_2CO_3, chloroform water or glycerin. In each case 1 cc. of the liquid was used for each gram of brain tissue.

As a check on this method and also for the sake of comparison with other methods the following procedures were carried out:

The ground tissue (with or without preservative) was spread in thin layers on watch glasses and placed in large desiccators. The glasses were surrounded by a cylindrical screen outside of which calcium chloride was placed. The desiccator was placed in a jar and packed with ice and salt. When the tissue was frozen the desiccator was evacuated. By frequent renewing of the vacuum the tissue could be dried in one to two days. A part of this dried material was tested directly while the rest was ex-

tracted twice with benzene by continual shaking in a mechanical shaker. After this treatment a light powdery substance was left. When this was used for enzyme study an amount of extractive and water was used that so compared with that used on the moist tissue that the enzyme content should be comparable.

The chief objection to this ideal method is that it cannot easily be used on large amounts of material and it is somewhat slow. A quicker method is to spread the ground tissue and preservative in very thin layers on glass plates. These plates were placed in a specially constructed box through which a current of air was drawn by an electric fan. By frequent turning, the tissue can be dried in ten to fifteen hours. Some of this air-dried material was treated with benzene to remove the lipoids, as had been done with the tissue dried by the freezing method.

In making the extractions the tissue was placed in cheesecloth sacks and suspended in wide mouth bottles containing the extractives. More preservative was added and the bottles stoppered. These were usually left at toom temperature for from three to seven days. The sacks were then lifted out of the extractives, allowed to drain for a few hours and discarded. The extracts were placed in a refrigerator and used as soon as possible.

The test solutions were ordinarily prepared as follows: 4 cc. of substrate were added to 10 cc. of extract and 100 cc. of water. The solution was mixed thoroughly and exactly 50 cc. placed in each of two 150 cc. flasks. One of these was boiled to destroy the enzymes, and both were then placed for 48 hrs. in an air thermostat kept at 37.5°. When a large number of tests were to be run on the same substrate, a solution was made containing the right proportions of substrate and water and 100 cc. of this solution used for 10 cc. of extract. In the case of insoluble substrates like olive oil and ethyl butyrate, emulsions of these were produced by making the liquids slightly alkaline and thoroughly shaking. When activators were used they were always added to the enzymes before the substrate had been put in.[1] After 48 hrs. in the thermostat the amount of activity was determined. For the action on fats, fat-like compounds and salol, direct titration was made with 0.1 *N* sodium hydroxide using phenolphthalein as indicator. For the action on proteins the formaldehyde method was used. With amylases the amount of activity was estimated by the copper hydroxide reduction test.

Results and Discussion. 1. Lipase.

Extractives for Lipase.—It was desirous at the outset to find the best method of extracting the enzymes, or at least a good method. It is obvious that the method which might prove best for one enzyme might be

[1] Rosenheim and Shaw-Mackenzie, *J. Physiol.*, 40, Pro. VIII (1910).

a very poor method for extracting others. Active lipolytic extracts[1] were obtained with all the solvents used.

Two such series after extracting for seven days gave the following results when acting on a mixture of equal amounts of monoacetin and monobutyrin:

TABLE I.

Tissue.	Extractive.	0.1 *N* NaOH required for neutralization.	
		Cc.	Cc.
Fresh cerebrum	H_2O	1.34	0.85
Fresh cerebrum	0.2% HCl	0.13	0.24
Fresh cerebrum	0.9% NaCl	1.21	0.82
Fresh cerebrum	0.5% Na_2CO_3	1.30	0.70
Fresh cerebrum	$CHCl_3$ water	1.34	1.13
Fresh cerebrum	Glycerin	(3.75)[2]	0.78
Frozen cerebrum, no preservative	H_2O	1.94	..
Frozen cerebrum, no preservative	Glycerin	(0.11)	..
Frozen cerebrum with preservative	H_2O	2.31	..
Air dried cerebrum	H_2O	1.37	..
Air dried cerebrum (ext. with benzene)	H_2O	2.71	..

Another series of extractions was run on the cerebrum with a view of finding out the rate of extraction of the various liquids. In this series ethyl butyrate was used as a substrate.

TABLE II.

Tissue.	Extractive.	0.1 *N* NaOH required for neutralization after extracting for			
		5 days. Cc.	7 days. Cc.	9 days. Cc.	12 days. Cc.
Cerebrum	H_2O	0.30	1.25	1.18	1.83
Cerebrum	0.2% HCl	0.19	(0.85)	0.50	0.57
Cerebrum	0.9 %NaCl	0.38	0.60	1.10	1.19
Cerebrum	0.5% Na_2CO_3	0.45	0.64	1.30	1.18
Cerebrum	Glycerin	0.15	0.14	0.37	0.50

The results of these experiments indicate that water, sodium chloride, sodium carbonate and chloroform water are about equal as extractives. The acid extractive seems to be less well suited for extracting the lipase, since the values for its activity are considerably lower. Probably the difference in the results with the various extracts of glycerin is due partly to the substrate used and partly to the amount of water present with the glycerin. It will be noticed that with the frozen dried tissue the amount of activity, when extracted with glycerin (no water being present), was

[1] J. H. Coriat, *Am. J. Physiol.*, **12**, 353 (1904); A. Pagenstecher, *Biochem. Z.*, **18**, 285 (1909); A. Wroblewski, *Compt. rend.*, **152**, 1334 (1911); L. Buetow, *Biochem. Z.*, **54**, 40 (1913).

[2] The values in parenthesis are not considered comparable and are obviously inconsistent with the other data given. Explanations for these apparent discrepancies will be given wherever possible.

very small. The value 0.78 agrees best with other data on the glycerin water extracts and is very likely the most reliable one for comparison.

The drying method in general seems to give slightly better results than the direct extraction method. The air-dried tissue extracted immediately with water gave about the same activity as the fresh tissue extracted with water, the figures being 1.34 and 1.37. The air-dried tissue which had been extracted with benzene gave the highest activity of any of the dried portions. Just why this is true is not clear, unless the lipoids exert an inhibiting or protecting influence upon the lipase.

With the frozen tissue extracted with water greater activity was obtained than with fresh tissue extracts. The tissue frozen without a preservative gave a little less activity than that in which a preservative was used. (A preservative was used in both cases when the extractions were made.) It is doubtful if these figures are of any great significance other than the fact that the preservative, which was oil of mustard in these series of experiments, exerted no inhibiting influence upon the enzyme.

Best Medium for Lipase.—With regard to the nature of the medium in which the lipase acts best, experiments show that a medium of slight acidity is best suited to its activity. In testing out this point, alkaline extracts were used in two different experiments. In one flask the solution was left alkaline, while in the other a slight acidity was produced. Similarly acid extracts were tried out in both acid and alkaline media. With a mixture of monoacetin and monobutyrin, the following results were obtained:

TABLE III.

	Cc. 0.1 *N* NaOH.	
	I.	II.
0.5% Na_2CO_3 extract; slightly acid.....	1.15	0.70
0.5% Na_2CO_3 extract; slightly alkaline.....	0.79	0.37
0.2% HCl extract; slightly acid.....	0.33	0.24
0.2% HCl extract; slightly alkaline.....	0.17	0.18

The alkaline extract as shown before is more active than the acid, but larger activity is produced by the alkaline extract if the medium is made slightly acid at the start. The acid extract likewise is better suited to an acid medium.

Substrates.—The lipase found in different portions of the brain possesses the power of acting on many substrates. Some results obtained with different extracts are given in Table IV.

There is no basis for comparison of the various extracts of this table since the conditions under which the different experiments were carried on were varied.

The best substrates are triacetin and monoacetin. Tributyrin, monobutyrin and ethyl butyrate were acted upon quite readily. Lard, tallow

and butter may owe their lesser activity to the fact that they do not go into solution and at best form only finely divided particles when ground with water in a mortar or thoroughly shaken with water. Cephalin and lecithin were affected to the same degree as olive oil. No specificity of action was noticed with brain lipase other than the one of rate of hydrolysis of the various substrates.

TABLE IV.

	Cc. of 0.1 *N* NaOH required for neutralization.			
	Water ext. of cerebrum.		Water ext. of medulla.	Na_2CO_3 ext. of cerebrum.
Substrate.				
Triacetin	..	..	0.56	0.36
Monoacetin	1.37	2.80	..	0.29
Tributyrin	..	..	..	0.24
Monobutyrin	0.84	2.40	0.25	0.08
Ethyl butyrate	..	2.23	0.15	0.13
Olive oil	0.15	0.26	0.29	(0.56)
Lard (hog)	..	..	..	0.19
Tallow (beef)	..	..	..	0.16
Butter	..	..	..	0.17
Lecithin	0.25	0.19	..	..
Cephalin	0.20	0.27	..	0.23

Conditions Best Suited for Action.—Since the activity at any one time depends upon a large number of conditions it was decided to run a set of experiments in which each condition in succession would be varied while the others would be constant. The different conditions varied were the amount of extract, the amount of substrate, the substance used as an activator, the amount of dilution, and the time during which the enzyme was allowed to act. The substrate used was ethyl butyrate.

In preparing the solutions in these experiments, a considerable amount of each proportion of ethyl butyrate and water desired was made slightly alkaline and thoroughly emulsified. When used, the emulsion was thoroughly mixed with the extract and two equal amounts of the mixture taken, one for a check and the other for enzyme activity. In the cases where an activator was used, it was always mixed with the extract before either was added to the substrate.[1] The figures in the results represent the cc. of 0.1 *N* NaOH required to neutralize the difference between the check and normal flasks.

Where one set of conditions was varied the remaining conditions were kept under circumstances found favorable in previous experiments. Thus, when the amount of extract was varied with the amount of substrate, no activator was used, the dilution was 50 cc. and the time two days. When the amount of dilution was varied with the time, 5 cc. of extract were used, 0.2 cc. of substrate, and no activator.

The results indicate clearly that the amount of activity of the brain

[1] Rosenheim and Shaw-Makenzie, *J. Physiol.*, 40, Pro. VIII (1910).

TABLE V.

Activators.

	Cc.	Substrate in cc. 0.1.	0.2.	1.	NaH_2PO_4 Na_2HPO_4.	Sodium glycocholate.	Saponin.	Dilution in cc. 0.	25.	100.	Time in days. 1.	3.	6.
Extract	2	0.19	0.28	0.41	0.22	0.12	0.20	0.28	0.09	0.10	0.07	0.31	0.60
	5	0.26	0.37	0.53	0.38	0.31	0.34	0.49	0.36	0.44	0.18	1.40	1.65
	15	0.40	0.64	1.25	0.55	0.20	0.60	(1.60)	0.44	0.68	0.28	1.60	2.15
Substrate	0.1				0.20	0.0	0.15	0.40	0.30	0.27	0.10	0.47	0.68
	0.2				0.30	0.41	0.34	0.53	0.44	0.49	0.19	0.52	1.03
	1.0				0.92	0.53	0.68	0.77	(1.30)	0.53	0.61	0.88	1.43
Activators. Na_2HPO_4 / NaH_2PO_4								(1.80)	0.57	0.60	0.25	0.58	1.25
Activators. Sodium Glycocholate								0.22	0.12	0.34	0.14	0.48	0.65
Activators. Saponin								0.35	0.34	0.50	0.22	0.63	0.97
Dilution	0										0.30	0.70	(0.68)
	25										0.18	0.45	1.05
	100										0.23	0.84	1.29

lipase on ethyl butyrate varies directly with the amount of enzyme, and the amount of substrate, used. In every case with the exception of one, the activity was increased where either one of these factors was enlarged. The one exception is the case of the variation of the amount of extract with sodium glycocholate as an activator. Here the greatest activity, contrary to expectations, was obtained in the flask containing 5 cc. of extract and not in the one containing 15 cc.

In varying the dilution a peculiar result was obtained. Activity was, as a rule, greater in both the smallest and largest dilutions than it was in the medium dilution. This may be accounted for by supposing that water inhibits the enzyme, but that the larger the dilution the oftener the interacting molecules collide.

In the flasks in which the time of action of the enzyme was varied, the amount of activity gradually increased with the time. With one exception, there was no evidence of the end point having been reached even at the conclusion of the sixth day.

With the activators the results were less definite. The amount of phosphates used was 2.5 cc. of a 1% solution of equal amounts of the monobasic and dibasic phosphates of sodium. 2.5 cc. of a 0.02 molar solution of sodium glycocholate, and 2.5 cc. of a 0.25% solution of saponin were used. The greatest activity was obtained with the phosphates and the least with the sodium glycocholate, although the activity was not increased markedly in any case. As is to be noticed from the table, it is doubtful if the activity was increased at all with sodium glycocholate. If any conclusion can be drawn it would seem to be that sodium glycocholate does not markedly increase the activity of brain lipase on ethyl butyrate under any of the conditions under which it was tested.

With saponin slightly higher activity was obtained, but this was not much above the normal activity. The three values obtained for saponin when the amount of extract was varied, were 0.20, 0.34, and 0.60. The three corresponding normal values were 0.28, 0.37, and 0.64. When the amount of substrate was varied, the three values were 0.15, 0.34, and 0.68, while the normal values were 0.26, 0.37, and 0.53. It would seem from these latter results that saponin has more influence in larger amounts of substrate, although the differences are not sufficient to guarantee this conclusion. The values obtained for the activity of saponin under different dilutions do not differ to any appreciable extent from the normal values. With the different lengths of time the values are also close to normal.

The values obtained with a mixture of mono- and dibasic phosphates acting on different amounts of enzyme are approximately equal to the normal values, and the amounts of activity obtained with the two smaller concentrations of substrate are not far from the normal values, but the value obtained with phosphates acting on a large amount of substrate is

considerably higher than the corresponding normal value, the figures being 0.92 and 0.53, respectively. This result would seem to indicate that phosphates, like saponin, increase the activity in large concentrations of the substrate. The results obtained for different lengths of time through which the enzyme was allowed to act are not markedly different from the values obtained when no phosphates were present.

In regard to the influence of activators on the action of the enzyme, it should be stated that larger increases in activity were obtained when monoacetin was used as a substrate with a water extract of the cerebrum. Values obtained under comparable conditions are as follows:

TABLE VI.

	Cc. 0.1 *N* NaOH.
5 cc. ext., 0.2 cc. monoacetin	2.87
5 cc. ext., 0.2 cc. monoacetin, 2.5 cc. 0.02 *M* sodium glycocholate	3.05
5 cc. ext., 0.2 cc. monoacetin, 2.5 cc. 0.25% saponin sol	3.11
5 cc. ext., 0.2 cc. monoacetin, 2.5 cc. 1.0% phosphate sol	3.63

From these results it will be seen that sodium glycocholate and saponin slightly increased the activity of the lipase, while phosphates increase it markedly. Previous investigators[1] have shown that saponin and sodium glycocholate considerably increase the activity of the pancreatic lipase. The accelerating action of phosphates on the brain lipase has also been reported.[2]

Lipase in the Different Divisions of the Brain.—The different divisions of the brain when kept under conditions as nearly as possible identical and extracted with water, gave the following activity:

TABLE VII.

	Cc. of 0.1 *N* NaOH.			Cc. of 0.1 *N* NaOH.	
	I.	II.		I.	II.
Cerebrum	1.34	1.25	Medulla	0.45	0.36
Cerebellum	(2.10)	0.87	Corpus callosum	0.61	0.56
Mid-brain	1.25	1.23			

These results indicate clearly that the gray matter is more active from a lipolytic standpoint than the white. The corpus callosum which is made up almost entirely of white matter would naturally be expected to give the lowest activity, but although it was obtained as free as possible from gray matter, it nevertheless showed greater activity than the medulla. The activities of the two cerebrums were about equal, while the activity of one cerebellum was higher and the other lower than those of the cerebrum and mid-brain. Very probably the lower value is nearly correct. Since the mid-brain contains large nuclei of gray matter, it seems probable that the greater part of its activity depends upon these. The medulla,

[1] Rosenheim and Shaw-Mackenzie, *J. Physiol.*, 40, Pro. VIII (1910).

[2] A. Wroblewski, *Compt. rend.*, **152**, 1334 (1911).

which is very similar to the mid-brain structurally but lacks the large nuclei, showed considerably less activity.

Lipase in Brains of Different Animals.—Human, beef, sheep, and dog brains have been tested for lipolytic activity and all found to be active. No experiments were run with extracts from each source under strictly comparable conditions, so that an exact estimate could not be made of the relative quantitites of lipase contained in the brain from the different sources. Figures which were obtained from extracts of the different brains acting on triacetin are given below:

TABLE VIII.

	Cc. 0.1 *N* NaOH.		Cc. 0.1 *N* NaOH.
Human cerebrum	3.50	Sheep cerebrum	4.31
Beef cerebrum	4.50	Dog cerebrum	3.60

While these values do not give an accurate idea of the lipase content of the different brains, they nevertheless show that all have the ability to split triacetin to about the same extent.

2. Proteolytic Enzymes.

The presence of a proteolytic enzyme[1] in nerve tissue has been indicated by the action of tissue extracts on various proteins. Time did not permit of an extensive study of this enzyme, or enzymes; but it has been found to attack several different proteins. A glycerin-water extract of the cerebrum prepared by the freezing method gave the following results when the resulting solution was tested for amino acids by the formaldehyde method:[2]

TABLE IX.

	Cc. 0.1 *N* $Ba(OH)_2$.		Cc. 0.1 *N* $Ba(OH)_2$.
Egg albumen	0.2	Witte's peptone	0.95
Gelatin	1.92	Nuclein	0.69
Casein	0.98		

The splitting of Witte's peptone was expected because of the presence of erepsin in most of the tissues so far studied; but it would seem that erepsin alone would hardly account for the large action on the more complex proteins, and it is probable that a pepsin or trypsin-like enzyme, or both, are present throughout the brain as well as in the hypophysis where both have been found.[3]

The proteolytic action of brain extracts on Witte's peptone takes place most rapidly in alkaline medium.

TABLE X.

Witte's peptone, slightly acid medium.....0.50 cc. 0.1 *N* $Ba(OH)_2$
Witte's peptone, slightly alkaline medium.....1.92 cc. 0.1 *N* $Ba(OH)_2$

[1] Kutscher and Lohman, *Z. physiol. Chem.*, **39**, 313 (1903); H. M. Vermon, *J. Physiol.*, **32**, 33 (1904); A. Wroblewski, *Compt. rend.*, **152**, 1334 (1911); L. Buetow, *Biochem. Z.*, **54**, 40 (1913).

[2] S. P. L. Sorensen, *Biochem. Z.*, **7**, 45 (1908).

[3] L. Buetow, *Biochem. Z.*, **54**, 40 (1913).

Tests of the various kinds of extracts on Witte's peptone gave the following amounts of activity, 5 cc. of extract and 5 cc. of a saturated solution of peptone being used in each case:

TABLE XI.

	0.1 *N* $Ba(OH)_2$.		0.1 *N* $Ba(OH)_2$.
Water	0.15	0.5% Na_2CO_3	0.25
0.2% HCl	0.12	$CHCl_3H_2O$	0.21
0.9% NaCl	0.16	$C_3H_5(OH)_3$	0.45

Here again the acid extract is shown to form a poor medium for the action of the protease. The sodium carbonate extract is better, but the highest activity was obtained with the glycerin extract and the results of a previous table show further that that extract was effective on other proteins as well as on Witte's peptone (Table IX).

3. Other Brain Enzymes.

In searching for *rennin*, negative readings were obtained by the use of the metacasein test.

The *amylase* content of brain tissue seems to be very slight.[1] No action at all could be detected in tests on starch, glycogen, amygdalin, sucrose, maltose, lactose, and dextrose. Dextrin, however, was slightly hydrolyzed. A solution of *dextrin* in which the enzyme had been allowed to act, gave a slight copper hydroxide test for the presence of a reducing sugar, while the check solution gave no test. The glucosides, arbutin and salol[2] are hydrolyzed by water extracts of the brain. A solution of *arbutin* after being acted upon by the enzyme gave a strong copper hydroxide test, while in the boiled check no copper was reduced.

With *salol* the following results were obtained by direct titration of the solutions:

TABLE XII.

	Cc. 0.1 *N* NaOH.
Unboiled flask containing enzyme and salol	1.52
Boiled flask containing enzyme and salol	0.76
Difference = enzyme activity	0.76

Since the cerebroside, cerebrin, contains a galactose grouping, it was thought possible that this might be split off by the amylase, but in several tests made on a mixture of cerebrosides no reduction of copper was obtained.

Catalase can easily be proved present in brain tissue. A water extract of the cerebrum was divided into two portions, one of which was boiled. Equal amounts of these two solutions were then mixed with equal amounts

[1] A. Wroblewski, *Compt. rend.*, **152**, 1334 (1911); Wohlgemuth and Szecsi, *Z. Neurol. Psych.*, 13, 454 (1913); L. Buetow, *Biochem. Z.*, 54, 40 (1913); V. Kafka, *Zentr. Biochem. Biophys.*, 14, 294 (1913).

[2] A. Wroblewski, *Compt. rend.*, **152**, 1334 (1911).

of hydrogen peroxide, placed in fermentation tubes and set in the thermostat. At the end of two hours the tube containing unboiled solution had in it from four to eight cc. of gas which proved to be oxygen, while the boiled check had produced no gas. The results show that the decomposition of hydrogen peroxide, which takes place at a slow rate under normal conditions, is greatly accelerated by the presence of the brain extract, and since such acceleration is not produced by boiled extract, it must be due to some thermolabile compound contained in the extract.

Tests were made for other enzymes and negative results obtained.

Peroxidase[1] was tested for by the guaiac test, in which guaiac, in the presence of hydrogen peroxide, is turned blue if the enzyme is present. No blue coloration was obtained. Tests for *oxidase* also resulted in the negative. A solution of alpha naphthol was not changed to a blue color in the presence of a tissue extract as is the case when certain oxidases are present;[2] formaldehyde was not oxidized to formic acid; and xanthin and hypoxanthin were not converted into uric acid. It is probable, since both of these enzymes have been reported by several investigators, that conditions were not exactly favorable for their detection or the specific tests were not used. It has been shown that glucose[3] and other powerful reducing substances, haemoglobin,[4] and protein interfere with different oxidase tests.

Reductase could not be demonstrated by the methylene-blue test which depends upon the decoloration of a dilute solution of methylene blue by formaldehyde. Here again the conditions may have been unfavorable for the action of the enzyme.

Guanase, which transforms guanine into xanthine, and *urease*,[5] which produces carbon dioxide and water from urea, could not be detected in the brain extracts. It is probable that both of these, if present at all, are present in such small quantities as to be undetectable by the methods used.

Summary.

1. The brain contains a lipase, erepsin, an amylase, a catalase, enzymes which decompose arbutin and salol, and probably a pepsin or trypsin-like enzyme, or both.

2. The lipase and proteases are best extracted by a mixture of glycerin and water or by water.

3. Extracts of tissue, dried either by the freezing or air-current method, showed greater activity than extracts of fresh tissue.

4. The highest activity was obtained from tissue extracted after drying with benzene to remove the lipoids.

[1] Levene and Stookey, *Biochem. Z.*, 13, 44 (1903).

[2] F. Traetta-Mosea, *Gazz. chim. ital.*, 43, II, 138 (1913).

[3] E. Sieburg, *Z. physiol. Chem.*, 86, 503 (1913).

[4] Czyhlarz and v. Furth, *Hofmeister's Beitr.*, 10, 358 (1907).

[5] L. Buetow, *Biochem. Z.*, 54, 40 (1913).

5. Triacetin and monoacetin form the best substrates for the action of the lipase. Lecithin and cephalin are also acted upon by it.

6. The amount of activity of the lipase, as has been shown for other enzymes, is increased directly with the amount of enzyme, the amount of substrate, and the time during which the enzyme is allowed to act.

7. The amount of activity of the lipase is increased by the presence of sodium glycocholate, saponin, or a mixture of the mono- and dibasic phosphates of sodium.

8. The lipase acts best in a slightly acid medium, the erepsin in an alkaline medium.

9. In general, the gray matter is more active than the white. Higher activity was obtained with extracts of the cerebrum, cerebellum, or mid-brain than with extracts of the medulla, or corpus callosum.

10. Human, beef, sheep, and dog brains all contain similar amounts of lipase.

11. Negative results were obtained in tests for peroxidase, oxidase, reductase, guanase, urease, and rennin.

URBANA, ILL.

NOTE.

The Origin of Petroleum.—It has been some time since I suspended publication on various lines of study on petroleum and its constituents, and it therefore seems necessary, to retain this field, that a brief mention be made of the present condition of my work, that others may not with perfect propriety take up some parts of it which I now have in progress. I am now using the information gained during the last thirty years in aid of further examination of the constituents of petroleum with especial reference to their original formation. It appears that twenty or more crude oils including those of Russia, South America, Texas, Canada and Louisiana contain the identical series of nitrogen compounds that were formerly described as present in California petroleum. These compounds are not pyridine as was formerly made plain in the paper on the California oils. This subject I have now in hand, together with a method for the determination of the small proportions of nitrogen contained in crude oils.

I am also carrying on a study of the action of sulfur on the constituents of petroleum, the series C_nH_{2n+2}, C_nH_{2n}, C_nH_{2n-2}, and C_nH_{2n-4}. Hydrogen is readily removed at temperatures near 150° with the formation of heavier oils, and at the same time with the formation of sulfur derivatives of the hydrocarbons. It is proposed to include in these changes the hydrocarbons all the way to the asphalts. I also have in hand series of hydrocarbons obtained by the distillation of coal *in vacuo*, and of gilsonite