to be present only in abnormal urines, according to Folin, it seems desirable to use sodium hydroxide rather than the sodium carbonate to free the ammonia. In general, it looks as though one is correcting an occasional appreciable error by introducing a frequent slightly smaller error.

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THE SYNTHESIS OF FATS BY THE ACTION OF ENZYMES.

By F. L. DUNLAP AND L. O. GILBERT.

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As a result of the various studies on the subject of lipolysis, it has been recognized that the reaction is never a complete one, the degree of hydrolysis depending on the conditions under which the experiments have been conducted. An hydrolysis of over 90 per cent. is not unusual, while on the other hand it may be considerably less, as for example, in the case of tributyrin when hydrolyzed by the lipase of the castor oil bean. Here Connstein, Hoyer and Wartenberg found but 9.5 per cent. hydrolysis after 24 hours.[1] In the latter case it is probable that no equilibrium is reached between the glyceride and the water and the products of hydrolysis, but rather that the acid produced either inhibits the lipolytic action when it reaches a certain concentration or the enzyme is destroyed.[2] The inhibition of fermentative processes by the normal products of such fermentation is well recognized, as in the case of lactic acid and alcoholic fermentation. That the lipolytic ferment, such as exists in the liver, pancreas, castor oil bean, etc., would not produce complete hydrolysis led to various successful attempts to synthesize fats through their action on mixtures of glycerol and the various fatty acids. Not only has the synthetic power of lipase been indicated, but of other enzymes as well.

With one exception experimental studies of synthesis due to lipase have been confined to this enzyme obtained from animal sources when acting on glycerol and the acids normal to fats. It is also worthy of note that many of these studies have been made with acids of exceptional occurrence in fats. This is seen, for example, in the work of Kastle and Loevenhart,[3] who studied pancreatic lipase and its synthetic effect on isobutyric acid and ethyl alcohol. Again, Hanriot[4] has produced monobutyrin by the action of glycerol on butyric acid by means of pancreatic

[1] *Ber.*, 35, 3988 (1902).

[2] Bradley [(*J. Biol. Chem.*, 8, 251 (1910)] is of the opinion that by increasing sufficiently the mass of the lipase, complete hydrolysis will ensue, but this is hardly in keeping with the views ordinarily expressed. For example, see Taylor [Univ. of California Publications, *Pathology*, 1, 35 (1904)].

[3] *Am. Chem. J.*, 24, 491 (1900).

[4] *Compt. rend.*, 132, 212 (1901).

lipase, while Pottevin[1] has produced mono- and di-olein by use of the same ferment. Taylor[2] has prepared triolein by the action of pancreatic powder, glycerol and oleic acid. Bodenstein and Dietz[3] studied the synthetic effect of pancreatic lipase on butyric acid and isoamyl alcohol. Pottevin[4] has also studied the effect of the pancreatic ferment on oleic acid and various alcohols, as methyl, ethyl, propyl, isopropyl, etc., with the result that esterification was obtained. Twitchell's naphthalenestearosulfonic acid which, as far as its properties are concerned, might be referred to as a synthetic enzyme, also has synthetic power, for in the absence of water it causes fatty acid and glycerol to condense.[5] Hamsik[6] has shown that a powder prepared from the mucous membrane of the small intestin of horses, sheep and hogs possesses synthetic power when applied to a mixture of oleic acid and glycerol. He has also shown that fat is synthesized through the action of the pancreatic lipase of the pig acting on palmitic and stearic acids and glycerol. The synthesis was identified by means of the decrease in the acid value.[7] A similar preparation from cattle and dogs possesses no such power.

Reference has been made to the one study recorded in the literature of the synthetic action of a vegetable lipase acting on glycerol and an acid normal to fat. As a result of this experimental work,[8] Taylor was able to synthesize triolein by means of the lipase of the castor oil bean. He mixed the glycerol with about one-fourth more than the molecular quantity of fatty acid necessary to produce the triglyceride. In all the tests the volume was 150 cc., to which was added 5 grams of the oil-free ricinus. After sealing, the flasks were shaken to emulsify. Controls were also used. After six months the flasks were opened and analyzed. The neutral fat produced was separated and the saponification number and iodine number determined. These were found to be, respectively, 185 and 81.2, sufficiently close to the theory to identify the product.

The work which we have to record was carried on several years ago and while its final results are similar to those obtained by Taylor, yet the method of proof is different and it has seemed worthy of permanent record. The work of Taylor was unknown to the authors at the time the experiments were conducted.

The enzyme used for this work was that found in the castor oil bean. It was deemed unnecessary to work with the very active cytoplasm as

[1] *Compt. rend.*, **136**, 767 (1903).
[2] *J. Biol. Chem.*, **2**, 102 (1906).
[3] *Z. Elektrochem.*, **12**, 605 (1906). Dietz, *Z. physiol. Chem.*, **52**, 279 (1907).
[4] *Ann. de l'Inst. Pasteur*, **20**, 901 (1906).
[5] THIS JOURNAL, **29**, 566 (1907).
[6] *Z. physiol. Chem.*, **59**, 1 (1909).
[7] *Ibid.*, **71**, 238 (1911).
[8] [Univ. of California Publications, *Pathology*, **1**, 33 (1904).]

described by Nicloux,[1] but the bean was, after decorticating, thoroughly ground and exhausted with ether and used in this form. Many experiments were conducted in order to find a suitable emulsifying agent, the one proving most satisfactory being flaxseed. When glycerol, oleic acid, flaxseed, and the oil-free castor bean are thoroughly ground together an emulsion is formed which will persist for days. It has been shown by one of us that the ungerminated flaxseed was devoid of lipolytic power,[2] which is amply shown by the blank test hereafter to be described. If this flaxseed possessed lipolytic power it would without question, in view of our present knowledge of the subject, also possess a certain degree of synthetic power. The blank experiment amply shows that it was not synthetic in the least.

In other experiments which have been published by one of us[3] it was found necessary to use preservative agents, such as chloral. In the present series of experiments, such agents were unnecessary as is shown in the blank experiments. Molds and bacteria do not thrive in the absence of water, or when water is present only in small amounts.

The method used was briefly as follows: Glycerol, oleic acid, flaxseed, and the oil-free castor oil bean, in a finely comminuted form, were thoroughly triturated in a mortar until an emulsion was formed, which was divided among several weighed flasks. An estimation of the acid was made at once in the contents of one of the flasks, the other flasks being reserved for the estimation of the acid value on succeeding days. The blank experiment was made in exactly the same way, except that the amount of oil-free castor bean was replaced by flaxseed. Considerable work was done before a method was found for getting concordant results in the estimation of the oleic acid in this emulsion. The method which proved satisfactory consisted in the treatment of the emulsion in the flask with ether, breaking up the emulsion thoroughly by means of a glass rod flattened at the bottom, and subsequent titration by means of a standardized alconolic potash. It will be seen from the following figures that the amount of oleic acid in the blank remained practically constant, whereas in the case of the two experiments where the castor oil bean was used, there was a marked and steady decrease in the amount of oleic acid. This decrease could only have been due to the esterification of the oleic acid. The fact that the castor bean enzyme has synthetic power is shown very clearly by these results and it did not seem necessary to attempt an isolation of the product in order to prove this point. These results do not show whether the combination of the oleic acid and the glycerol produce a mono- or di-glyceride before the triglyceride is

[1] *Compt. rend.*, **138**, 1112, 1288, 1352 (1904); **139**, 143 (1904).

[2] Dunlap and Seymour, THIS JOURNAL, **27**, 935 (1905).

[3] *Ibid.*, **27**, 940 (1905).

produced, but judging from the results obtained by Pottevin and Hanriot, whose work has been discussed, it is possible that the interaction between the glycerol and the oleic acid takes place in three stages. It is, however, worthy of note that Taylor isolated only triolein.

In the experimental data which follow, the material for preparing the emulsion was weighed on a rough balance and in each of the three studies recorded the following substances were used:

5 grams oil-free castor bean.
5 grams flaxseed.
25.5 grams glycerol.
16.7 grams Kahlbaum's oleic acid.

In the case of the blank, over 10 grams of flaxseed were used and no castor oil bean. The glycerol was used quite largely in excess of the amount theoretically necessary to esterify the oleic acid.

Blank experiment.

Time.	Milligrams oleic acid in 1 gram of the mixture.
At once	315.5
After 2 days	319.0
After 4 days	315.7
After 6 days	314.8
After 8 days	315.5
After 11 days	314.9

Experiment using castor oil bean.

Time.	Milligrams oleic acid in 1 gram of the mixture.
At once	337.3
After 2 days	322.0
After 3 days	320.2
After 6 days	311.6
After 9 days	290.5
After 11 days	248.6

This shows a combination of 88.7 milligrams of oleic acid with glycerol, that is, a disappearance of over 26 per cent. of the total oleic acid present.

Another experiment, however, which did not run so long as the one recorded above, gave the following data:

Time.	Milligrams oleic acid in 1 gram of the mixture.
At once	302.7
After 2 days	287.2
After 4 days	257.6
After 7 days	230.8

There seems little doubt but that the decrease in the free acid would have been greater had the experiment been carried on for a longer period. Unfortunately it was impossible to extend this work at that time. The

results here reported, as well as those obtained by Taylor, show undoubtedly that the enzyme of ricinus has synthetic power.

Since this article was written there has appeared in the *Z. angew. Chem.*, **24**, 385 (1911) an article by Adolf Welter. He discusses some experimental work on the reversibility of enzyme action and gives some results of the synthesis of fats from fat acids and glycerol through the action of the castor oil bean ferment. Welter uses blanks identical with mixtures used, only heated to 60–80° to destroy the activity of the lipase. He recognizes the change brought about by the decrease in acid values.

The experimental work on this subject was performed at the University of Michigan.

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THE STABILITY OF THE PHOTOGENIC MATERIAL OF THE LAMPYRIDAE AND ITS PROBABLE CHEMICAL NATURE.[1]

BY F. ALEX MCDERMOTT.

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One of the oldest and at the same time one of the most important observations relative to the chemical processes involved in the production of light by living creatures, is that the photogenic tissues may be dried, preferably in the absence of air (oxygen), and preserved, again out of contact with air (oxygen), for considerable periods, without losing the power to evolve light when moistened in the presence of air (oxygen). This fact, which seems so remarkable at first sight, appears to have been discovered by Reaumur in his work on the luminous bivalve *Pholas dactylus*, in 1733. The observation was repeated by Spallanzani in 1794 and 1796, by Carradori in 1798, by Carus in 1864, and by Dubois in 1886. Spallanzani extended it to the luminous *Medusae*, Carus to the fireflies (*Lampyridae*), and Dubois to the *Myriapoda* and the *Elateridae*. Pflüger, in 1875, seems to have regarded the luminous tissue of the *Lampyridae* as of too little vitality to exhibit this phenomenon, but the recent work of Kastle and McDermott on this same subject indicates that his conclusion was probably based on material dried in the air.

Kastle and McDermott showed that if the luminous organs of the common firefly of this region, *Photinus pyralis*, were dried *in vacuo* with a residual atmosphere of hydrogen, and subsequently sealed *in vacuo* or in hydrogen, the tissue will retain its photogenic power, and exhibit

[1] Presented at the 44th Annual meeting of the American Chemical Society, Section on Biological Chemistry, Indianapolis, Ind., June 28, 1911.