

“The Hydrolysis of Fats *in vitro* by means of Steapsin.” By Dr. J. LEWKOWITSCH and Dr. J. J. R. MACLEOD, Mackinnon Scholar. Communicated by Professor E. DIVERS, F.R.S. Received May 11,—Read May 28, 1903.

A few months ago, one of us (J. L.) stated\* that he had made a series of experiments in which lipase was allowed to act on cotton-seed oil, and that he had only been able to obtain hydrolysis amounting to 3 per cent. Dr. Macleod then suggested that it would be of considerable interest to ascertain the extent to which steapsin could carry the hydrolysis under the same conditions. We, therefore, decided to investigate this question conjointly, and we are now in a position to show beyond doubt that steapsin is capable of hydrolysing (saponifying) fats outside the organism to a very great extent. As this fact appears to us of considerable physiological importance, inasmuch as the quantitative experiments have hitherto been made almost entirely on monobutyryn and simple esters,† we publish this preliminary notice without referring to the literature *in extenso*. This will be done after the completion of the experiments we have in hand.

#### *Preparation of the Steapsin Solution.*

The steapsin solutions A and B (see table) were prepared from fresh ox pancreas. The pancreas was freed of fat, minced, and 200 grams ground up in a mortar with water. In the case of preparation A, 0·1 per cent. of mercuric chloride was added, and in B some thymol. The preparations were then digested in the incubator at body temperature for 20 hours.‡ Next they were filtered, and the filtrate examined for steapsin by vigorously shaking 2—3 c.c. in a test tube with a few drops of filtered butter fat, adding a drop of an alcoholic solution of phenolphthalein and then decinormal caustic soda solution, till a deep red colour was obtained. Exactly the same mixture was

\* ‘Jour. Soc. Chem. Ind.,’ 1903, p. 67.

† Nencki (“Spaltung der Säureester der Fettreihe und der aromatischen Verbindungen im Organismus und durch das Pancreas,” ‘Arch. für exper. Path. u. Pharmak.’ vol. 20, p. 367) tested the action of an aqueous extract of pancreas on mutton fat, but found hydrolysis to proceed only to about 20 per cent. By adding bile to the digest the saponification amounted to about 60 per cent. Pawlow (‘The Work of the Digestive Glands,’ translated by W. H. Thompson, London, 1902) also records determinations by Dr. Walther (pp. 29, 39) of the steatolytic action of pancreatic juice obtained from a fistula, but the results do not give us an estimate of the actual amount of hydrolysis effected. They were used by the workers for comparative observations.

‡ These are the methods recommended by Ferd. Klug (‘Ueber Gasentwicklung bei Pankreasverdauung,’ ‘Pflüger’s Archiv,’ vol. 70, p. 329, 1898).

placed into a second test tube, the pancreatic extract having, in this case, been heated to destroy its ferments. The preparations were incubated at 37° C. After about half-an-hour the preparations were examined. In the case of the steatolytically active mixture, the contents of the test tube were found to be discoloured, and the amount of decinormal caustic soda solution necessary to restore the original red tint was ascertained. Preparations C and D were obtained from 200 grams minced, fresh pig pancreas by simply triturating in a mortar with twice the bulk of water. In these cases the preparations were not incubated, for it was evident that in the previous experiments with A and B the steapsin had been destroyed, as the preparations, before incubation, were very active steatolytically when tested by the above method, but only very slightly so after incubation; probably this was due to the action of trypsin on steapsin. It was also noticed in preparations A and B that the steapsin remained on the filter paper when the solutions were filtered, the filtrates having much weaker steatolytic powers than the precipitates. Preparations C and D were, therefore, only filtered through muslin.

#### *Preparation of the Emulsion of Fat and Steapsin.*

100 grams of cotton-seed oil, or lard, were weighed out and carefully triturated in a mortar with measured quantities of the pancreatic extract. Great care was taken to obtain a complete emulsion. The emulsion was then poured into bottles which were well corked to exclude growth of fungi, and allowed to stand at the ordinary temperature.\* If the temperature was raised too rapidly, say by immersion in a water bath kept at 30° or 35° C, the emulsified mass would separate into two layers, and no hydrolysis would then take place. It is therefore of the greatest importance to carefully observe the mixture for some hours, and to thoroughly shake them up as soon as signs of separation are noticed, in order to restore the state of emulsion. A good plan is to immerse the well-shaken emulsion in cold water, or to let it stand in the open during the night. After a few days, distinct hydrolysis is noticeable. In the case of cotton-seed oil, the outward sign of hydrolysis is the hardening of the mass, due to the fatty acids that have been formed.

#### *Consideration of Results.*

The following table contains a series of observations made on cotton-seed oil and lard. The amount of hydrolysis was measured by the percentage of free fatty acids, expressed in terms of oleic acid. The

\* Most of the preparations kept free from infection during the time these experiments lasted. Only a few became covered with growth of *Penicillium glaucum* after the lapse of about four weeks.

experiments with A B and C were started on February 19th, and those with D on March 5th. The first observations were made after a lapse of 4 days. The delay was caused through some of the emulsions having separated into two layers, which necessitated the re-establishing of the state of emulsion by frequent shaking. It will be noticed that in those experiments with cotton-seed oil, in which neither acid nor alkali had been added, hydrolysis had reached after four days, in the case of preparation C, from 22·9 per cent. (No. 5) to 32·8 per cent. (No. 6), and in the case of preparation D, from 31 per cent. (No. 11) to 37·1 per cent. (No. 9). After another 7 days, samples Nos. 11 and 9 had reached 46·3 per cent. and 44·3 per cent. respectively. As it was not expected that further hydrolysis would proceed very rapidly, some time was allowed to elapse before the next tests were taken. It will be seen from the table that the highest percentages of hydrolysis, when neither acid nor alkali was added, were 86·7 per cent. in the case of preparation C (No. 6), and 83·8 per cent. in the case of preparation D (No. 10). In both cases the steapsin had been allowed to act for 56 days. Whereas the experiments made with C show that the amount of hydrolysis increases with the amount of steapsin mixed with the oil, no such striking regularity is apparent in the case of preparations D (Nos. 9 to 11).

Further experiments were made with cotton-seed oil under the same conditions, only with that difference that either dilute acid or dilute caustic soda was added. So far, no decisive influence of either acid or alkali has been noticed, and from the experiments recorded here, no definite conclusions can be drawn.

Curiously enough, the hydrolysis of lard proceeds at a very much slower rate, reaching only about one-third of the hydrolysis noticed in the case of cotton-seed oil. Since the consistency of lard favours the state of emulsion, one would have expected the opposite result. The last two experiments with lard seem to show that an increased amount of caustic soda, whilst favouring hydrolysis at the commencement, seems later on to retard the action of the steapsin, notwithstanding the larger amount of the latter.

The numbers recorded in the table show that the steapsin is not capable of producing the reversible reaction which it was thought, reasoning by analogy, this enzyme might produce.

These preliminary experiments are very interesting from a physiological point of view; they prove for the first time that it can be demonstrated by the usual quantitative methods of fat analysis that steapsin is a very powerful fat-splitting ferment.

We are now investigating the action of steapsin and of lipase on fats, in the presence of bile, small quantities of soaps, and a number of other substances which suggest themselves from a physiological point of view.

No.	Preparation.	Cubic centimetres of preparation per 100 grammes of cotton-seed oil or lard.	Date on which started.	Added to emulsion.		Percentage of free fatty acids formed.					
				Acid.	Alkali.	Feb. 23.	Mar. 2.	Mar. 9.	Mar. 16.	Apr. 16.	May 1.
<i>Cotton seed-oil.</i>											
1	A 1	10	19/2/03	0	0	..	..	0.95	Separation into two layers had taken place.		
2	A 2	20	19/2/03	0	0	..	..	0.8	..	..	..
3	A 3	16	19/2/03	0.5 c.c. of 2 % acetic acid	0	..	..	1.14	..	..	..
4	B	10	19/2/03	0	0	..	..	0.95	..	..	..
5	C 1	20	19/2/03	0	0	..	..	43.3	48.4	80.6	..
6	C 2	30	19/2/03	0	0	22.9	..	49.9	60.45	86.7	..
7	C 3	25	19/2/03	0.5 c.c. of 2 % acetic acid	0	32.8	..	41.04	48.8	74.7	..
8	C 4	30	19/2/03	0	0.5 c.c. 1/10 norm. NaOH	10.22	26.4	55.4	63.1	..	..
9	D 1	30	19/2/03	0	0	31.25	39.5	37.1	44.3	68.3	70.2
10	D 2	50	5/3/03	0	0	..	..	32.7	46.4	71.5	83.8
11	D 3	60	5/3/03	0	0	..	..	31.03	46.3	60.8	79.5
12	D 4	60	5/3/03	0.5 c.c. of 2 % acetic acid	0	..	..	30.4	45.2	65.3	73.7
13	D 5	60	5/3/03	0	0.5 c.c. 1/10 norm. NaOH	..	..	36.5	44.9	66.4	73.5
14	D 6	30	5/3/03	0	1 c.c. 1/10 norm. NaOH ..	..	..	31.4	43.8	74.1	74.3
15	D 7	50	5/3/03	1 c.c. of 2 % acetic acid ..	0	..	..	37.66	44.5	65.2	77.2
<i>Lard.</i>											
16	D 8	50	5/3/03	0	0.5 c.c. 1/10 norm. NaOH	..	..	8.98	9.2	29.9	46.7
17	D 9	80	5/3/03	0	1 c.c. 1/10 norm. NaOH ..	..	..	12.2	14.4	20.7	22.9