CXXXII. THE EFFECT OF FAT ON THE TRYPTIC DIGESTION OF PROTEIN *IN VITRO*¹.

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THAT fat retards the digestion of protein in the body is an established fact (Luciani [1913], Pawlow and Thompson [1910], Cowie and Mumson [1908], Carlson [1914, 1915], Lockwood and Chamberlin [1923], Frank [1923]), but an explanation of this action has not yet been given.

It has been the aim of the following work to investigate one part only of the chemical aspect of the subject and not to reproduce the conditions of digestion in the alimentary canal. Further, protein was digested *in vitro* in the presence of a proteolytic enzyme free from any lipase, and thus no digestion products of fat were involved.

METHOD.

A solution of egg-albumin, p_H 1.7, was digested with pepsin for 24 hours at 37°, as such treatment accelerated the subsequent digestion with trypsin. Tryptic digestion was carried out in *M*/10 buffer solution of sodium phosphates, starting at p_H 8.0, in the presence of an emulsion of olive oil and controlled by a similar experiment in which the oil was omitted. The rate of tryptic digestion was determined by Sørensen's method.

Peptic digestion.

A solution was prepared containing

15 % egg-albumin (dried).

3 % pepsin (pepsin B.P. Allen and Hanbury's).

This was brought to p_H 1.7 by means of hydrochloric acid heated to 37° and was incubated at this temperature for 24 hours. Sodium hydroxide was added until the p_H reached the isoelectric point of denatured albumin [Chick and Martin, 1911, 1912].

It was heated for 30 minutes over a bath of boiling water; cooled for 15 minutes by means of running water and filtered through a Buchner funnel. This solution of the substrate was kept saturated with toluene and at a p_H of 5.5. The amounts of total nitrogen and, in the later work, the amino-acid

¹ An abridgment of a thesis for which the degree of Master of Science of the University of London was awarded.

nitrogen present in the solution of the substrate were estimated by the Kjeldahl method and Sørensen's method respectively, and from these results the amount of nitrogen available for digestion was determined.

Tryptic digestion and oil experiments.

Tryptic digestion was carried out in $M/10$ buffer solution of sodium phosphates. Liquor Trypsin Co. (Allen and Hanbury's) was used and olive oil as fat. The volume of $N/10$ NaOH was determined, for each sample of olive oil, which was required to bring 100 g. of oil in 100 cc. of absolute alcohol to that definite pink colour in the presence of phenolphthalein which was used as an end-point in later titrations.

Emulsion of oil. 1 part by weight of finely powdered gum acacia and 4 parts olive oil were mixed thoroughly in a mortar and then 2 parts of water were added, and the mixture stirred briskly until a permanent emulsion was obtained. The emulsion could be diluted with the substrate solution without separation of oil. Trypsin was added to the buffered solution of substrate until the resulting liquid contained 10 % of the former, and then NaOH until the p_H of the liquid was 8.0.

To equal volumes of buffered solution of substrate and trypsin (p_H 8.0) were added, in the emulsion experiment, the required weight of emulsion and in that of the control a weight of gum equal to that which was present in the emulsion, dissolved in such a volume of water as to bring this solution of gum to the same volume as that of the emulsion. In each case the liquids were saturated with toluene.

Two alternative methods were then employed.

A. The control mixture was brought to p_H 8.0 by the addition of NaOH solution and an equal volume of NaOH solution was added to the emulsion mixture.

B. Both control mixture and emulsion mixture were brought to p_H 8.0.

The capillator method of determining p_H was used.

The emulsion and control mixtures were brought in equal times and with constant stirring to 37° and incubated at that temperature in a water-bath for as long as the experiment lasted.

The amount of digestion was determined by Sørensen's method of direct titration in the presence of formaldehyde. A sample was taken before digestion began both from the emulsion mixture and the control and then from each at subsequent intervals during the experiment. These samples were titrated with $N/10$ NaOH until a definite pink colour was produced in the presence of phenolphthalein (p_H 8.8), and this volume, less that required before digestion, was taken as representing the degree of digestion which had occurred. It was decided that production of acidity as well as of amino-acids could be taken as an indication of the degree of digestion [cf. Lannoy, 1918].

The maximum error of this method was shown to be 0.52 cc. on a reading of 20 cc. whilst the average error was 0.08 cc. on that volume.

The following curves show the results of typical experiments:

Fig. 1. Digestion curves (experiment 33).

SUMMARY OF RESULTS.

Method A. In those cases in which the control mixture was brought to p_H 8.0 by the addition of alkali and an equal volume of alkali was added to the emulsion mixture, no definite proof that oil in the form of an emulsion has any effect on the digestion *in vitro* was obtained, as the variations which occurred were almost all within the experimental error.

It is interesting to note, however, that the lower percentages of oil were insufficient to alter the p_H of the emulsion mixture perceptibly (see Fig. 2), but in the presence of more than 13 % of oil the p_H of the emulsion mixture was 7.9 before digestion began, whilst that of the control was 8.0 (see Fig. 1) and that it became higher than the control after 1 hour's digestion and remained so throughout. Even so, as the result of 39 experiments, no definite increase in digestion was evident.

In every one of these experiments, however, a small initial retardation in the presence of oil was noted followed by a later acceleration (see Figs. 1

and 2). Though such variations from the control were practically all within the experimental error, the phenomenon was so regularly obtained that it is felt that the work would be worth repeating with a still more sensitive method of p_H estimation.

Method B. On the other hand, if the emulsion mixture as well as the control were brought to p_H 8.0 the emulsion experiments showed an increased amount of digestion (6 experiments). The p_H of the emulsion mixture became higher than that of the control from the first hour and remained so throughout the experiment (see Fig. 3).

Fig. 2. Digestion curves (experiment 38).

DISCUSSION OF RESULTS.

In connection with the foregoing results certain factors may be involved.

(1) In those experiments in which the control and emulsion mixtures were brought to p_H 8.0 before digestion, oil in the form of an emulsion exerted a small accelerating effect on tryptic digestion. This acceleration increased as digestion proceeded, but never amounted to more than 10 %. This is probably connected with the fact that the p_H of the emulsion mixture never

fell as low as that of the control mixture. Sørensen has shown that trypsin is very sensitive to any change in hydrogen ion concentration.

Further, oil in the form of an emulsion may exert some action on the substrate. It was noticed that on the addition of alkali to the control mixture a flocculent precipitate appeared, but that in samples of the emulsion mixture which had been allowed to stand only a fine precipitate was evident. This fact, according to the observations made by Fodor [1922], would account for an increase in digestion in the emulsion mixture.

Fig. 3. Digestion curves (experiment 45).

Oil in the form of an emulsion may exert some protective action on trypsin itself. Northrop [1922] has shown that the normal action of trypsin is retarded by an inhibitor produced in the course of digestion. It is possible that oil may protect trypsin from the action of this substance, and also from the action of alkali.

(2) In those cases, however, in which the control mixture was brought to p_H 8.0 and an equal volume of alkali added to the emulsion mixture no definite effect of olive oil was observed, but a higher p_H was recorded in such concentrations of oil in which variations in p_H could be appreciated. As trypsin is very sensitive to any change in hydrogen ion concentration, it follows that

fat probably exerts some retarding action to balance the higher p_H of the emulsion mixture. Such a retarding action would most likely have entered into the experiments discussed under heading (1), but in these cases the retarding action was not sufficient to equalise the digestion in emulsion mixture and control.

It has been shown that surface plays a very important part in enzyme action. Oil reduces surface tension and so lowers surface energy, hence a retardation in digestion would be expected. Meyerhof [1914] has shown that the inhibiting effect of alcohols on enzyme action varies as their power to lower surface tension.

Bayliss [1911] obtained a similar result. He found that saponin reduced surface energy and had thus a retarding action on the effect of urease on urea. He [1906] also demonstrated the fact that adsorption takes place between a substrate and enzyme. It is possible that the presence of fat may affect the rate of formation of this adsorption compound.

At the present stage it is impossible to say how far each of these factors is concerned, but the gross results of the foregoing experiments show that olive oil, unhydrolysed and in the form of an emulsion, does not retard the action of trypsin *in vitro* on albumin previously digested with pepsin. The explanation of the retarding action of fat on the digestion of protein *in vivo* may be found either in the action of the products of the hydrolysis of fat or in some specific action of fat in the alimentary canal.

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REFERENCES.

- Bayliss (1906). *Biochem. J.* **1**, 175.
— (1911). *Proc. Roy. Soc. Lond. B*, **84**, 81.
Carlson (1914). *Amer. J. Physiol.* **36**, 153.
— (1915). *Amer. J. Physiol.* **38**, 248.
Chick and Martin (1911). *J. Physiol.* **43**, 13.
— (1912). *J. Physiol.* **45**, 264.
Cowie and Mumson (1908). *Arch. int. Med.* **1**, 64.
Fodor (1922). *Kolloid Z.* **31**, 280.
Frank (1923). *Wilm. Arch. inn. Med.* **5**, 591.
Lannoy (1918). *Compt. Rend. Soc. Biol.* **81**, 72.
Lockwood and Chamberlin (1923). *Arch. int. Med.* **31**, 96.
Luciani (1913). *Human Physiol.* **2**, 178.
Meyerhof (1914). *Pflüger's Arch.* **157**, 251.
Northrop (1922). *J. Gen. Physiol.* **4**, 227, 261.
Pawlow and Thompson (1910). *The Work of the Digestive Glands*, 119, 142