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THE EFFECT OF FAT ON THE TRYPTIC DIGESTION OF PROTEIN IN VITRO.

T5.1095-

That fat retards the digestion of protein in the body is apparently established.

Aristotle appreciated this property of fat for he observed "Now the best digestion is in the bottom of the stomach, because the fat descends on there; such as those that eat fat meat are very sleepy by reason that digestion is hindered."

Luciani (1) states that in the digestion of meat, collogen is digested first, then muscular fibrils and parenchymatous cells and finally tissue impregnated with fat.

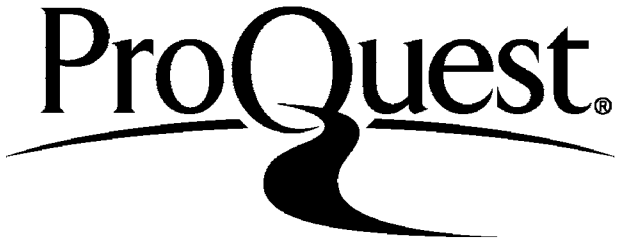
Pawlow in his classic work on dogs (2) found that olive oil delayed gastric secretion, but "acted as an independent exciter on the pancreatic gland". (3) Carlson confirmed many of Pawlow's results and worked on human subjects. (4) (5) Cowie and Muhson (6) substantiated the work of Pawlow. They found :-

1. That olive oil given with a meal decreased the gastric acidity at the end of an hour, and retarded evacuation of the stomach.

2. That the beginning of secretion of hydrochloric acid was delayed when oil preceded a meal and unchanged when oil followed it.

3. That the maximum digestion was delayed when oil was given either before or after a meal, that is to say, the ascending part of the digestion curve was not so steep.

Lockwood and Chamberlain (7) confirmed the first of these observations. Frank (8) also found that olive oil given before a meal led to a reduction in the average acidity, though he showed that fat given with a meal had no influence on the secretion of



of acid unless the fat were present in a hydrolysed state. On the other hand Rehfuss' results show that any reduction in the acidity of the contents of the stomach leads to an earlier evacuation of this organ (9), hence it would appear that the retardation of the evacuation of the stomach found by Cowie and Hunsen was not the result of the reduction of acidity in the stomach, but was due to some other specific action of the oil.

The process of digestion involved a number of factors and much work has already been contributed. Bayliss' work is well-known from his joint paper with Starling (10) establishing the presence of a chemical stimulus for the secretion of pancreatic juice up ~~the~~ to the end of his more recent work on the physical aspect of enzyme action and digestion. (11 to 19). Contributions concerning the movements of the alimentary canal, a factor connected with the rate of digestion, have been put forward by Cannon who advanced a method of investigation of these movements by Röntgen Rays (19, 20, 21). He also published work concerning the effect of nervous factors on the rate of digestion (22).

Thus it is evident that the presence of fat may influence many factors in digestion. It may interfere with the rate of secretion of the digestive juices or it may coat the protein so that an intimate connection between the enzyme and substrate is not possible. This latter action would not only delay digestion directly, but would delay the production of the chemical stimulus for further secretion of enzyme.

In view of the complexity of the problem involved in the further study of the effect of fat on the digestion of protein, 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.

The work was carried out in vitro, thus eliminating the physiological factors of digestion. Protein was digested in the presence of a proteolytic enzyme free from any lysase. Thus only the presence of fat on the digestion of protein was studied and no digestion products of fat were involved. Commercial preparations of the enzymes were used and the experiments carefully controlled.

When the work was begun trypsin was used as the ferment and blood fibrin as the substrate. Fibrin was obtained from a cattle market and dried in an air oven. The ninhydrin colorimetric method, due to Harding, was adopted for the estimation of amino acid nitrogen produced during the digestion. In this method a solution of triketo-hydrindene hydrate in the presence of pyridine is added to the solution to be tested, and the colour of the resulting mixture compared in a colorimeter with an equivalent mixture in which the solution to be tested is replaced by an equal volume of a standard alanine solution. It was found that the sample of fibrin used gave a yellowish tint, which made it impossible to match the digested liquid against the standard. The method was finally abandoned,

and Sorensen's method of direct titration in the presence of formaldehyde was adopted.

Instead of fibrin a solution of dried egg albumin was used to remove the uncertainty of the introduction of equal surfaces of fibrin in different experiments. Tryptic digestion, however, proved to be so very slow (22) that the albumin was previously digested with pepsin in the presence of hydrochloric acid at PH=2.0. This method had the added advantage of similarity to the conditions of digestion of egg albumin in the alimentary canal. Even so, titrations of only 4 to 6 cc $\frac{N}{10}$ NaOH were obtained with a 4% solution of albumin, as representing the tryptic digestion over a period of five hours. In order to produce an adequate substrate for tryptic digestion a series of peptic digestion experiments were carried out in which the concentration of egg albumin was varied, then that of pepsin and later the PH. Tryptic digestion experiments were made on successive days with this digestion mixture as substrate and finally conditions and proportions were found which would give adequate titrations during tryptic digestion and these were adopted in the subsequent work.

FINAL METHOD OF WORK.

1. Peptic Digestion.

A solution was prepared containing:-

15% egg albumin (dried)
3% Pepsin. (Pepsin B.P. Allen & Hanbury's).

This was brought to PH = 1.7. by means of hydrochloric acid, and the liquid heated to 37°C over water. It was incubated at that temperature in a water bath for 24 hours. Caustic Soda was then added to the liquid until the PH - 5.5 i.e. the isoelectric point of denatured albumin. (7) (8).

It was heated for 30 minutes over a bath of boiling water; cooled for 15 minutes by means of running water; and, finally filtered through a Buchner funnel. Thus a clear yellowish liquid (the solution of the substrate) was obtained, which would keep satisfactorily in the cold room. The stock was kept saturated with toluene and at a PH = 5.5.

Peptic digestion was originally carried out at PH = 2.0. Then the mixture was boiled to inactivate the ferment, and also to congulate any excess albumin. After being cooled, the liquid was filtered and kept in the cold room. In this way a homogeneous, but opague, liquid was produced, whose capacity to support tryptic digestion decreased with the length of time that it was kept in stock. It was noticed that precipitation occurred in this stock solution, and the amount of precipitation increased with keeping. This meant that not only did the

titrations of tryptic digestion decrease, but the precipitate introduced a source of error, so the solution, which had been digested with pepsin, was brought to PH = 5.5 before heating. This procedure resulted in a maximum coagulation and a clear filtrate, which was kept at PH = 5.5, and which produced practically no precipitation on being kept.

The amounts of total nitrogen and in the later work the amino acid nitrogen present in the solution of the substrate were estimated by the Kjeldahl Method and Sorensen's Method respectively, and from these results the amount of nitrogen available for digestion was determined.

2. Tryptic Digestion and Oil Experiments.

Tryptic digestion was carried out in a $\frac{M}{10}$ buffer solution of sodium phosphates. Liquor trypsin Co. (Allen & ~~Allen~~Hanbury's) was used as the ferment and Olive Oil as the fat. The volume $\frac{M}{10}$ Caustic Soda was determined for each sample of olive oil which was required to bring 100 gms 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.
4 " " " " olive oil.

were mixed thoroughly in a mortar, and then 2 parts by weight of water were added, and the mixture stirred briskly until a permanent emulsion was obtained. The emulsion could be diluted

with the solution of the substrate without separation of the oil. Trypsin was added to the buffered solution of substrate until the resulting liquid contained 10% of the former, and then caustic soda until the PH of the liquid was 8.0.

To equal volumes of the buffered solution of substrate and trypsin (PH - 8.0) were added, in the case of an emulsion experiment, the required weight of emulsion (the resulting liquid being referred to as the emulsion mixture) 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 (the resulting liquid being referred to as the control mixture). In each case the liquids were saturated with toluene. Then two alternative methods were employed:-

(a) The control mixture was brought to a PH = 8.0 by the addition of caustic soda, and equal volume of caustic soda added to the emulsion mixture.

At this time the comparator method of determining PH was used, but although it was practicable for the control mixture the emulsion mixture was much too opaque to allow of its use. Hence it was necessary to bring the control mixture to PH = 8.0. and add an equal volume of alkali to the emulsion mixture thus correcting for the presence of the gum. Later when the capillator method (due to Henry A.Ellis) was produced, it was

possible to estimate the PH of the emulsion mixture so treated colorimetrically, and it was found that this mixture was slightly less alkaline than the controls if the former contained more than 10% oil. In the presence of lower percentages of oil, this method of determination of P.H. did not indicate any difference between the control and emulsion mixtures in the presence of an $\frac{M}{10}$ buffer solution.

(b) Both control mixture and emulsion mixture were brought to PH = 8.0.

This was rendered possible by the use of the capillator method for determination of PH. In this colour standards are contained in sealed capillary tubes, prepared from alkali free glass and mounted on a card. The liquid to be tested and indicator are pipetted into a similiar tube and matched against the standards. Although an error is introduced by the dilution of the liquid to be tested with an equal volume of the solution of indicator, this error is the same for the control and emulsion experiment, and therefore is cancelled.

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

The amount of digestion in each case was determined by Sorensen's method of direct titration in the presence of formaldehyde. 25 cc of the digesting mixture were withdrawn and delivered into a tube containing 30 cc of formaldehyde solution.

This solution contained one part by volume of formalin to two parts of water and was made pink to phenol-phthalein by the addition of caustic soda. Redistilled formalin was used as it was found that some of the commercial samples gave a yellow colour in the presence of alkali, which colour disappeared on standing. Such a sample of the digesting mixture was taken before digestion began from both the emulsion mixture and the control mixture, and then from each at subsequent intervals of time during the experiment.

The contents of the tubes were titrated with $\frac{N}{10}$ NaoH until a definite pink colour was produced in the presence of phenol-phthalein (PH = 8.8). By substracting the titration values of those samples of the control mixture and emulsion mixture in which no digestion had occured from subsequent samples of each respectively, it was possible to decide the degree of digestion which had occurred.

Originally a sample of 25 cc of the digesting mixture was delivered into a tube containing acid, which stopped all tryptic digestion. Caustic soda was added until the liquid was pink to phenol phthalein (PH - 8.8.) and then the required volume of formaldehyde solution. This mixture was titrated with $\frac{N}{10}$ NaoH until the pink colour was restored. Later experiments were done with the samples of trypsin used in this work, to ensure that there was no lipase present. As no sample of trypsin contained such a ferment, it was decided that the acidity as well as the

amino acids produced could be taken as an indication of the degree of digestion which had taken place. Lannoy (9) has shewn that acidity produced during digestion of protein is a good indication of the degree of digestion, and that a curve representing the rate of production of acidity is similar to that representing the rate of production of free amino acids.

Experiments were carried out to determine the error of this method. A solution of buffered substrate and trypsin was digested and two samples of 25 cc withdrawn at definite intervals and mixed with 30 cc of formaldehyde solution (as above). To one sample was added the experimental proportion of emulsion and to the other a weight of gum, equal to that contained in the emulsion, dissolved in water so that the volume of the gum solution was equal to the volume of the emulsion. The maximum error of this experiment was shewn to be 0.4cc whilst the average of the whole 15 titrations embodied in the table was 0.08 cc. (see table 1.)

TABLE 1.

RESULTS OF EXPERIMENTS IN WHICH THE EMULSION AND GUM SOLUTIONS WERE ADDED TO THE TRYPTIC DIGEST AFTER DIGESTION AND BEFORE TITRATION.

		CONTROL.		EMULSION.		
No.	Time Incubated.	Volume $\frac{N}{10}$ NaOH used to titrate 25 cc of digestion mixture in the presence of formaldehyde.	Ditto corrected for titration of sample in which no digestion has occurred.	Volume of $\frac{N}{10}$ NaOH used to titrate 25 cc. of digestion mixture in the presence of formaldehyde.	Ditto corrrected for titration of sample in which no digestion has occured.	Control corrected titration less emulsion corrected titration
1.	8 hrs.	48.79 cc.	0 cc.	48.79.	0	0
	1	59.41 "	10.62 "	59.43.	10.64.	-0.02.
	2.5. mins.	63.92. "	15.13.	64.31.	15. 52.	-0.39.
	3 "	66.67. "	17.88.	66.78.	17.99.	-0.11.
	4 "	68.89. "	20.10.	68.59.	19.80.	0.30.
	5 "	70.74. "	21.95.	70.21.	21.42.	0.52.
2.	8.	46.79. "	8.	47.49.	0.	0.0.
	1.	57.07. "	10.28.	57.76.	10.27.	0.01.
	2.	61.67. "	14.88.	62.62.	15.13.	-0.25.
	3.	64.18. "	17.39.	65.07.	17.58.	-0.19.
	4.	66.00. "	19.21.	66.30.	18.81.	0.40.
	4.45.	67.40. "	20.61.	67.69.	20.20.	0.41.
2.	8.	47.04. "	0.	47.92.	0.	0.
	1.	54.47. "	10.43.	57.95.	10.03.	0.40.
	2.	60.87. "	13.83.	61.81.	13.99.	-0.16.
	3.	64.18. "	17.14.	64.74.	16.82.	0.32.
	4.	66.55. "	19.51.	67.13.	19.21.	0.30.
	5.	68.10. "	21.06.	68.90.	20.98.	0.08.

The exact way in which the experiments were carried out will be seen from the following details.

Details of a Typical Experiment. (33) Group (a). See Table II and Graph II.

14.58% oil was used and PH determined by the comparator method.

To 540 cc of buffered substrate (PH = 8.0) was added 60 cc of trypsin. 10 cc of the mixture was withdrawn and titrated with $\frac{N}{10}$ NaOH to PH = 8.0. 2.5 cc were required and therefore 13.35 cc N. NaoH were added to the remaining 590 cc.

I. Control Mixture.

Fifteen grams of Gum dissolved in a sufficient quantity of water, so that the volume of gum solution was 110 cc. were added to 290 cc of the above mixture as PH = 8.0. } volume 400.

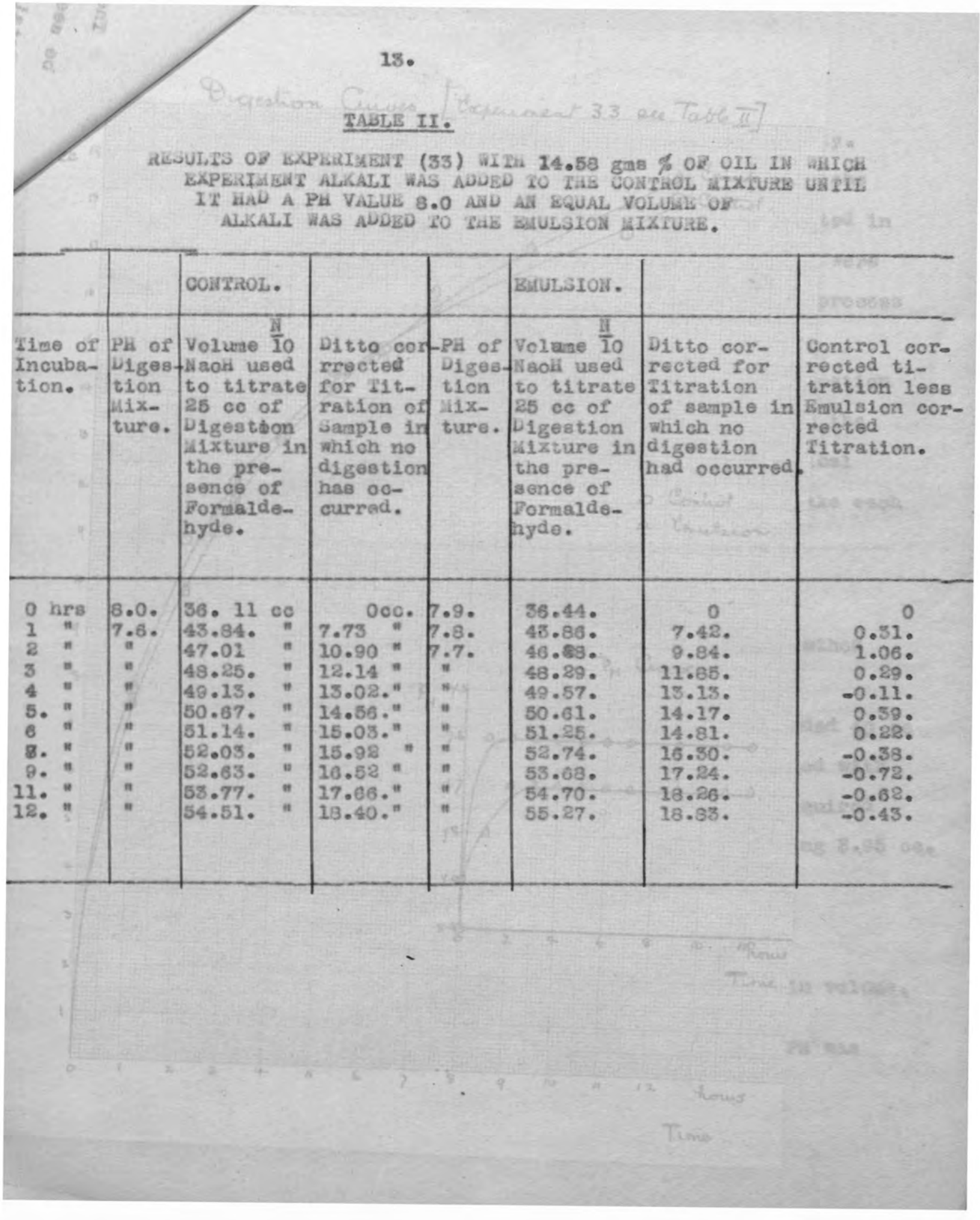
10 cc of this mixture were withdrawn and titrated with $\frac{N}{10}$ NaoH to PH = 8.0. .0.5cc were needed and therefore 1.97cc N. NaoH were added to the remaining 390cc of liquid. The PH was checked by the capillator method and 8 cc of toluene were added.

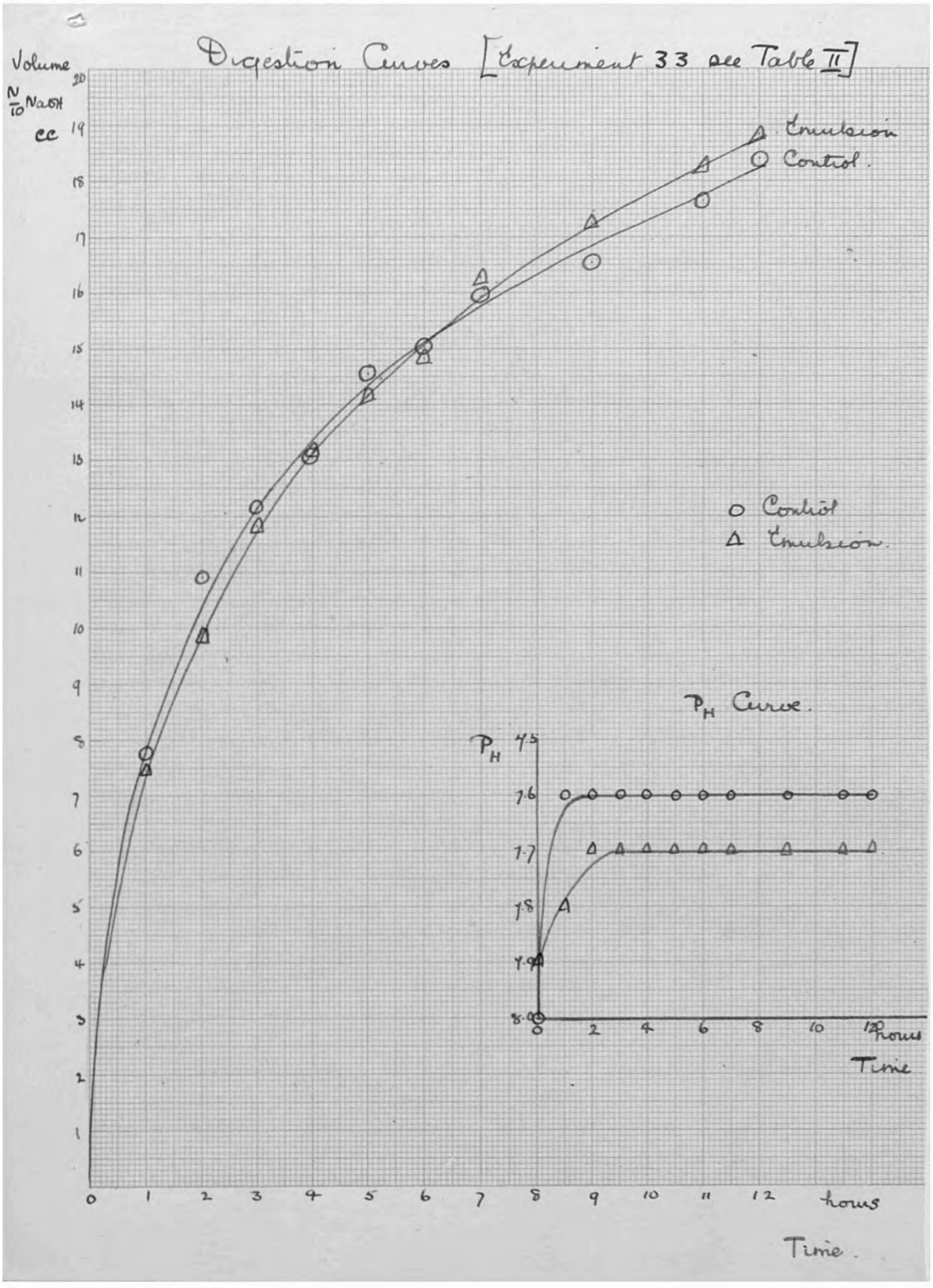
The control mixture was warmed to 37.0°C with constant stirring over a heated wire gauge. The gas was lighted about 5 minutes before it was required, so as to ensure a steady supply of heat. It took three minutes to bring this liquid to the required temperature, and the flask was lifted slightly off the

TABLE II.

RESULTS OF EXPERIMENT (33) WITH 14.58 gms % OF OIL IN WHICH EXPERIMENT ALKALI WAS ADDED TO THE CONTROL MIXTURE UNTIL IT HAD A PH VALUE 8.0 AND AN EQUAL VOLUME OF ALKALI WAS ADDED TO THE EMULSION MIXTURE.

		CONTROL.			EMULSION.		
Time of Incubation.	PH of Digestion Mixture.	Volume $\frac{N}{10}$ NaoH used to titrate 25 cc of Digestion Mixture in the presence of Formaldehyde.	Ditto corrected for Titration of Sample in which no digestion has occurred.	PH of Digestion Mixture.	Volume $\frac{N}{10}$ NaoH used to titrate 25 cc of Digestion Mixture in the presence of Formaldehyde.	Ditto corrected for Titration of sample in which no digestion had occurred.	Control corrected titration less Emulsion corrected Titration.
0 hrs	8.0.	36. 11 cc	0cc.	7.9.	36.44.	0	0
1 "	7.8.	43.84. "	7.73 "	7.8.	43.86.	7.42.	0.31.
2 "	"	47.01 "	10.90 "	7.7.	46.88.	9.84.	1.06.
3 "	"	48.25. "	12.14 "	"	48.29.	11.85.	0.29.
4 "	"	49.13. "	13.02."	"	49.57.	13.13.	-0.11.
5. "	"	50.67. "	14.56."	"	50.61.	14.17.	0.39.
6 "	"	51.14. "	15.03."	"	51.25.	14.81.	0.22.
8. "	"	52.03. "	15.92 "	"	52.74.	16.30.	-0.38.
9. "	"	52.63. "	16.52 "	"	53.68.	17.24.	-0.72.
11. "	"	53.77. "	17.66."	"	54.70.	18.26.	-0.62.
12. "	"	54.51. "	18.40."	"	55.27.	18.83.	-0.43.




Digestion Curves [Experiment 33 see Table II]
Volume
N/10 NaOH
cc
Emulsion
Control.
Control
Emulsion.
PH Curve.
PH
7.5
7.6
7.7
7.8
7.9
8.0
0
2
4
6
8
10
12 hours
Time
hours
Time.

gauze, if the former was inclined to get heated too quickly. Then 25 cc. were withdrawn and the flask put at once into the incubating bath at 37.0°. The emulsion mixture was treated in the same way, care being taken that exactly three minutes were spent over the heating process and that throughout this process the base of the flask never became very hot.

Every time a sample was withdrawn from these digestion mixtures, it was found necessary to remove the flask from the bath and shake it, because precipitation occurred: a typical sample could not be got otherwise. Care was taken to shake each flask an equal number of times (10) and that rapidly.

Details of a Typical Experiment (38) Group (A).

5.433 gms of oil were used, and the capillator method adopted for the final titration.

To 360 cc of buffered substrate solution were added 40 cc of the trypsin solution. 5 cc were withdrawn and titrated with $\frac{N}{10}$ NaoH. until the mixture had a PH=8.0. 1.3 cc were required for this and, therefore, 10 cc were added to the remaining 3.95 cc. The total ~~xxxxxxxx~~ resulting volume was 405 cc.

1. Control Mixture.

200 cc of above liquid.
3.18 gms gum dissolved in water and equal to 70 cc in volume.
8.0 cc N. NaoH which brought the mixture to PH=8.0.

This volume was added little by little until the right PH was obtained.

II. Emulsion Mixture.

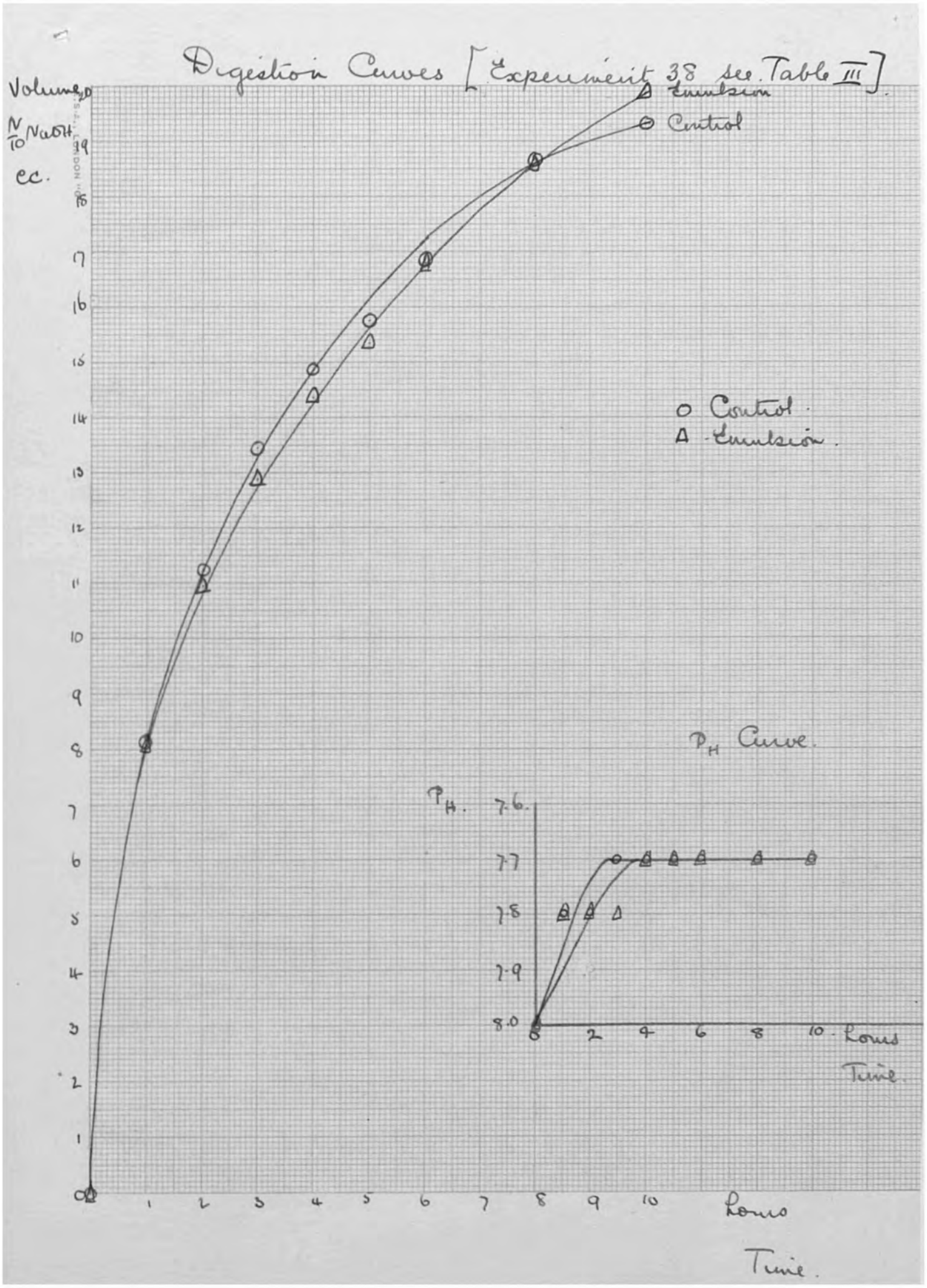
200 cc of above liquid.
21.9 gms. emulsion.
8.0 cc. N. NaoH.

Whenever a sample of the control mixture was withdrawn to estimate the PH during titration an equal volume was withdrawn from the emulsion mixture, and similarly, whenever alkali was added to the control mixture, an equal volume was added to the emulsion mixture. Further method was the same as that employed in experiment (32) see p. 12.)

TABLE III.

RESULTS OF EXPERIMENT (38) WITH 5.433 G.M.S. % OF OIL, IN WHICH EXPERIMENT ALKALI WAS ADDED TO THE CONTROL MIXTURE UNTIL IT HAD A P.H. VALUE = 8.0 AND AN EQUAL VOLUME OF ALKALI WAS ADDED TO THE EMULSION MIXTURE.

		CONTROL.			EMULSION.		
Time of Incubation.	PH of Digestion mixture.	Volume of $\frac{N}{10}$ NaoH used to titrate 25 cc of Digestion Mixture in the presence of Formaldehyde.	Ditto corrected for titration of Sample in which no Digestion had occurred.	PH of Digestion Mixture.	Volume $\frac{N}{10}$ NaoH used to titrate 25cc of Digestion Mixture in the presence of Formaldehyde.	Ditto corrected for Titration of Sample in which no Digestion had occurred.	Control corrected titration less Emulsion corrected Titration.
CONTROL							
0 hr.	8.0.	33.67.	0.	8.0.	33.76.	0.	0.
1 "	7.8.	41.82.	8.15.	7.8.	41.80.	8.04.	0.11.
2 "	7.8.	44.87.	11.20.	7.8.	44.74.	10.98.	0.22.
3 "	7.7.	47.12.	13.45.	7.8.	46.65.	12.89.	0.56.
4 "	7.7.	48.50.	14.83.	7.7.	48.09.	14.33.	0.50.
5 "	"	49.44.	15.77.	"	49.07.	15.31.	846.
6. "	"	50.51.	16.84.	"	50.50.	16.74.	0.10.
8 "	"	52.30.	18.63.	"	52.28.	18.52.	0.11.
10 "	"	52.98.	19.31.	"	53.62.	19.86.	-0.55.


Digestion Curves [Experiment 38 see. Table III].
Volume
N/10 NaOH
cc.
Emulsion
Control
Control.
Emulsion.
PH Curve.
PH.
7.6.
7.7
7.8
7.9
8.0
Hours
Time.
Hours
Time.

Details of a Typical Experiment (45) Group (B).

(See Table IV. and Graph III).

The capillator method for determination of PH was used throughout.

To 360 cc of the buffered substrate solution were added 40 cc of the trypsin solution.

I. Control Mixture.

200 cc of the above liquid.
9.4 gms of gum dissolved in water and equal to 70 cc in volume.
3.55 cc 4N NaoH, which brought the mixture to PH =8.0.
0.7 cc water to correct for additional alkali used in the case of the control.
5.5 cc. toluene.

II. Emulsion Mixture.

200 cc of the above liquid.
65.8 gms. emulsion.
4.25 cc 4N. NaoH which brought the mixture to PH = 8.0.
5.5 cc toluene.

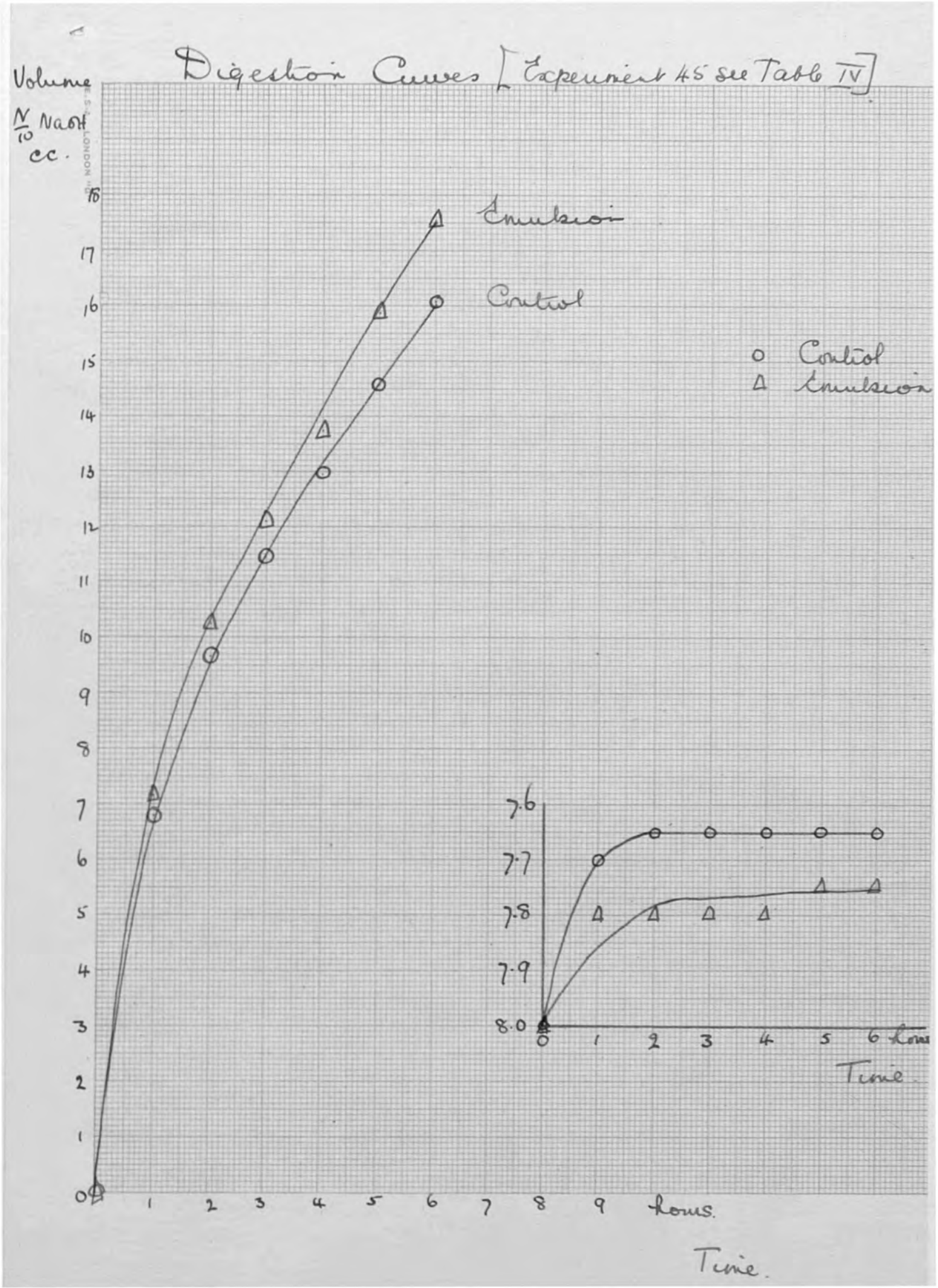
Further method was the same as that employed in experiment (32) (see p. 12)

N.B. At this stage of the work it was found most satisfactory to correct the PH once only.

TABLE IV.

RESULTS OF EXPERIMENT (45) WITH 13.82 GMS % OF OIL.
IN THIS EXPERIMENT BOTH CONTROL MIXTURE AND EMULSION MIXTURE WERE BROUGHT TO P.H.=8.0.

		CONTROL.			EMULSION.		
Time of Incubation.	PH of Digestion Mixture.	Volume $\frac{N}{10}$ NaoH used to Titrate 25 cc of Digestion Mixture in the presence of formaldehyde.	Ditto corrected for Titration of Sample in which no Digestion had occurred.	PH of Digestion Mixture	Volume $\frac{N}{10}$ NaoH used to titrate 25 cc of Digestion Mixture in the presence of formaldehyde.	Ditto corrected for Titration of Sample in which no Digestion had occurred.	Control corrected Titration less emulsion corrected Titration
CONTROL							
0 hrs.	8.0.	33.54.	0.	8.0.	31.41.	0.	0.
1 "	7.7.	40.33.	6.79.	7.8.	38.59.	7.18.	-0.39.
2 "	7.65.	43.20.	9.66.	"	41.66.	10.25.	-0.59.
3 "	"	45.01.	11.47.	"	43.51.	12.10.	-0.63.
4 "	"	46.52.	12.98.	"	45.12.	13.71.	-0.73.
5 "	"	48.11.	14.57.	7.75.	47.23.	15.82.	-0.25.
6. "	"	49.62.	16.08.	"	48.92.	17.51.	-1.43.


Digestion Curves [Experiment 45 see Table IV]
Volume N/10 NaOH cc.
Emulsion
Control
Control
Emulsion
0 1 2 3 4 5 6 7 8 9 hours.
Time.
7.6
7.7
7.8
7.9
8.0
0 1 2 3 4 5 6 hours
Time.

SUMMARY OF RESULTS OF THE OTHER EXPERIMENTS.

GROUP A. in which equal volumes of alkali were added to Control and Emulsion Mixtures.

No.	Total N. in Solution of Substrate.	Approximate Amount of Oil.	Length of Time of Tryptic Digestion.	Control Titration minus Emulsion Titration.
1.	gms. 0.432%	2.5.	1 hr.	0.03 cc.
		2	2 " 5 m.	0.26 "
			3 "	0.25 "
2.	"	"	1 hr. 3 m.	0.47 "
			2 " 3 "	-0.28 "
			3 " 3 "	0.16. "
			4 " 3 "	0.15 "
3.	"	"	1 "	1.29 "
			2 "	0.28 "
			3 "	0.12 "
			4 "	0.93 "
4.	"	"	55 m.	0.20 "
			2 hrs.	0.06 "
			3 "	0.23 "
			5 "	0.39 "

SUMMARY OF RESULTS OF THE OTHER EXPERIMENTS.

GROUP A. in which equal volumes of alkali were added to Control and Emulsion Mixtures.

No.	Total N. in Solution of Substrate.	Approximate Amount of Oil.	Length of Time of Tryptic Digestion.	Control Titration minus Emulsion Titration.
5.	GMS. 0.629%	GMS. 3.0%	1 hr.	-0.07 cc.
			2 "	0.24 "
			3 "	0.55 "
			4 "	0.22 "
			5 "	0.55 "
6.	"	"	1 "	-0.33 "
			2 "	0.55 "
			3"	0.39 "
			4"	0.28 "
			5 "	0.22 "
7.	Accident with Kjeldehl experiment.	"	1 "	0.40 "
			2 "	0.15 "
			3 "	-0.15 "
8.	"	"	1 "	0.33 "
			2 "	-0.17 "
			3 "	-0.28.

SUMMARY OF RESULTS OF OTHER EXPERIMENTS.

GROUP A. in which equal volumes of alkali were added to Control and Emulsion Mixtures.

No.	Total N. in Solution of Substrate.	Approximate amount of oil.	Length of Time of Tryptic Digestion.	Control Titration minus Emulsion Titration.
9.	0.447 gms.%	3.0 gms. %	1 hr.	0.06.
			2 "	0.36.
			3 "	0.63.
			4 "	0.78.
10.	" "	"	1 "	0.12.
			2 "	0.05.
			3 "	0.31.
			4 "	0.52.
11.	0.635 "	"	1 "	-0.40.
			2 "	-0.31.
			3 "	0.44.
			4 "	0.39.
			5 "	0.71.
12.	" "	"	1 "	-0.30.
			2 "	-0.46.
			3 "	0.06.
			4 "	0.38.
			5 "	0.05.
13.	0.665 "	"	1 "	0.02.
			2 " 8 mins.	0.29.
			3 "	0.34.
			4 "	0.49.
			5 "	0.97.
14.	"	"	1 "	0.23.
			2 "	0.62.
			3 "	0.19.
			4 "	0.49.

SUMMARY OF RESULTS OF THE OTHER EXPERIMENTS.

GROUP A. in which equal volumes of alkali were added to Control and Emulsion Mixtures.

No.	Total N. in Solution of Substrate.	Approximate Amount of Oil.	Length of Time of Tryptic Digestion.	Control Titration minus Emulsion Titration.
15.	0.665 gms %	3.0 gms %	1 hr.	0.20.
			2 "	0.06.
			3 "	0.03.
			4 "	-0.07.
			5 "	-0.04.
16.	"	"	1 "	0.06.
			2 "	0.06.
			3 "	0.03.
			4 "	0.00.
			5 "	0.03.
17.	0.619	"	1 "	0.03.
			2 "	0.06.
			3 "	0.25.
			4 "	-0.03.
			5 "	0.32.
18.	"	"	1 "	0.10.
			2 "	-0.13.
			3 "	0.16.
			4 "	0.11.
			5 "	0.58.
19.	0.682	"	1 "	0.06.
			2 "	0.10.
			3 "	-0.16.
			4 "	-0.07.
			5 "	0.09.
20.	"	"	1 "	0.29.
			2 "	0.79.
			3 "	0.20.
			4 "	0.69.
			5 :	0.25.

SUMMARY OF RESULTS OF THE OTHER EXPERIMENTS.

GROUP A. in which equal volumes of alkali were added to Control and Emulsion Mixtures.

No.	Total N. in Solution of Substrate.	Approximate Amount of Oil.	Length of Time of Tryptic Digestion.	Control Titration minus Emulsion Titration.
21.	0.634 gms %	3.0 gms %	1 hr.	-0.44 cc.
			2 "	-0.19.
			3 "	-0.09.
			4 "	-0.08.
			5 "	0.08.
22.	0.663 "	"	1 "	0.06.
			2 "	-0.23.
			5 "	-0.06.
23.	"	"	1 "	0.55.
			2 "	0.38.
			5 "	0.06.
24.	0.423	"	1 "	0.39.
			2 "	0.41.
			3 "	0.34.
			4 "	0.10.
			5 "	0.26.
25.	"	"	1 "	0.10.
			2 "	0.44.
			3 "	0.47.
			4 "	0.51.
			5 "	0.57.
26	"	5.0%	1 "	0.62.
			2 "	0.27.
			3 "	-0.28.
			4 "	1.09.
			5 "	0.56.

SUMMARY OF RESULTS OF THE OTHER EXPERIMENTS.

GROUP A. in which equal volumes of alkali were added to Control and Emulsion Mixtures.

No.	Total N. in Solution of Substrate.	Approximate Amount of Oil.	Length of Time of Tryptic Digestion.	Control Titration minus Emulsion Titration.
27.	0.423 gms. %	5.0 gms %	1 hr.	0.12 cc.
			2 "	0.84.
			3 "	0.83.
			4 "	0.85.
			5 "	1.03.
28.	0.956.	"	1 "	0.25.
			2 "	0.58.
			3 "	0.54.
			4. "	0.06.
			5 "	0.53.
29.	"	"	1 "	0.40.
			2 "	0.51.
			3 "	0.17.
			4 "	0.61.
			5 "	0.94.
37.	1.312.	"	1 "	0.55.
			2 "	0.05.
			3 "	0.31.
			4 "	-0.22.
			5 "	-0.31.
			6 "	-0.51.
			7 "	-0.12.
			8.15 mins.	-0.79.
38.	Quoted in full.			
39.	1.312.	5.0 gms. %	1 "	0.16.
			2 "	0.15.
			4 "	0.23.
			5 "	0.26.
			6 "	-0.34.
			8.11 mins.	-0.14.

SUMMARY OF RESULTS OF THE OTHER EXPERIMENTS.

GROUP A. in which equal volumes of alkali were added to Control and Emulsion Mixtures.

No.	Total N. in Solution of Substrate.	Approximate Amount of Oil.	Length of Time of Tryptic Digestion.	Control Titration minus Emulsion Titration.
30.	1.009 gms %	15 % Oil.	1 hr. 2 " 3 " 4 " 4.55 mins.	0.16 cc 0.25. 0.30. 0.48. 0.07.
31.	"	"	1 " 2 " 3 " 4 " 4.55 mins.	0.81. 0.08. 0.32. 1.19. 1.20.
32.	"	"	1 hr. 2 " 3 " 4 " 5 "	0.24. 0.04. 0.68. 0.42. 0.12.
33.	Quoted in full.			
34.	1.564 "	"	1 hr. 2 " 3 " 4 " 6 " 7 " 8 " 9.7 mins. 10.30 "	0.75. 0.47. 0.21. 0.03. 0.42. 0.19. 0.16. -0.33. -0.16.

SUMMARY OF RESULTS OF THE OTHER EXPERIMENTS.

GROUP A. in which equal volumes of alkali were added to Control and Emulsion Mixtures.

No.	Total N. in Solution of Substrate.	Approximate Amount of Oil.	Length of Time of Tryptic Digestion.	Control Titration minus Emulsion Titration.
35.	1.549 gms %	15 % Oil.	1 hr.	0.22.
			2 "	0.10.
			3 "	0.33.
			4 "	0.01.
			5 "	-0.36.
			6.2 mins.	-0.25.
			7 "	0.12.
			9.45 "	-0.34.
36.	"	"	1 hr.	0.04.
			2 "	0.33.
			2.55 mins.	0.24.
			4 hr.	-0.12.
			5 "	0.21.
			6 "	0.11.
			6.53 "	-0.08.
			9	-0.48.

GROUP (B) in which Control Mixture and Emulsion were both brought to PH = 8.0. Approximately 15% of Oil was used in the Emulsion Mixtures.

NO.	Total N in Solution of Substrate.	Length of time of Tryptic Digestion.	Control Titration minus Emulsion Titration.
40.	1.312 gms. %	1 hr.	-0.30.
		2 "	-0.61.
		3 "	-0.50.
		5 "	-0.81.
		7 "	-0.71.
		9 "	-0.68.
41.	"	1 "	-0.26.
		2 "	-0.58.
		3 "	-0.76.
		4 "	-0.20.
42.	"	1 "	0.33.
		2 "	0.60.
		3 "	0.84.
		4 "	0.85.
		5 "	0.01.

GROUP (B) In which Control Mixture and Emulsion Mixture were both brought to PH = 8.0. Approximately 15% of Oil was used in the Emulsion Mixtures.

No.	Total N in Solution of Substrate.	Length of Time of Tryptic Digestion.	Control Titration minus Emulsion Titration.
43.	0.996.	1 hr.	-0.76.
		2 "	-1.53.
		2.35 mins.	-1.58.
		3.35 "	-1.97.
		4.35 "	-1.81.
		5.35 "	-2.06.
44.	"	1 hr.	-1.54.
		2 "	-1.31.
		3 "	-1.54.
		4 "	-1.74.
		5.2 mins.	-1.28.
		6	-1.44.
		7.9 "	-1.41.
		8	-1.42.
		10.24 "	-1.51.

SUMMARY OF RESULTS.

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

It is interesting to note, however, that later observations showed that the lower percentages of oil were insufficient to alter the PH of the emulsion mixture perceptibly, but in the presence of more than 13% oil the PH of the emulsion mixture was 7.9 before digestion began, whilst that of the control was 8.0 and that it became higher than the control after one hour's digestion and remained so throughout (see Table II and III and graphs I and II). Even so, no definite increase in digestion was evident. Thirty-nine experiments in all were done.

In everyone of these experiments, however, a small initial retardation in the presence of oil was noted followed by a later acceleration (see graph I and II). 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 PH estimation.

(b) On the other hand, if the emulsion mixture as well as the control mixture was brought to PH= 8.0 so far as experiments

have been done the emulsion experiment showed an increased amount of digestion. The PH of the emulsion mixture became higher than that of the control from the first hour, and remained so throughout the experiment (see Table IV and Graph III). All these experiments were done with about 15% of oil.

DISCUSSION ON 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 PH = 8.0 before digestion, oil in the form of an emulsion exerted a small accelerating effect on tryptic digestion, in vitro, of egg albumin, previously digested with pepsin. This acceleration increased as digestion proceeded but never amounted to more than 10%. This is propably connected with the fact that although the control and emulsion mixtures were brought to the same PH before being submitted to digestion, the PH of the emulsion mixture never fell as low as that of the control mixture. Sorensen (31) has shewn that trypsin is very sensitive to any change in Hicn 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 (32) would account for an increase in digestion in the emulsion mixture.

Oil in the form of an emulsion may exert some protective action on trypsin itself. Northrop (33 and 34) has shewn 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 PH = 8.0 by the addition of alkali added to the emulsion mixture, no definite effect of olive oil in the form of an emulsion on the rate of digestion of protein was observed, but a higher PH was recorded in such concentrations of oil in which variations in PH could be appreciated. As trypsin is very sensitive to any change in Hion concentration, it follows that fat probably exerts some retarding action to balance the higher PH 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 (30A) has shown that the inhibiting effect

of alcohols on enzyme action varies as their power to lower surface tension. Bayliss (30b) obtained a similar result. He found that saponia reduced surface energy and had thus a retarding action on the effect of urease on urea.

Bayliss (35) has demonstrated the fact that absorption takes place between a substrate and enzyme. It is possible that the presence of fat may effect the rate of fumation of this absorption 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 and that the explanation of the retarding action of fat on the digestion of protein must be sought either in the action of the products of the hydrolysation of fat or in some specific action of fat in the alimentary canal.

SUMMARY.

1. A solution of dried egg albumin was digested with pepsin and used as the substance for tryptic digestion experiments in the presence of an emulsion of olive oil. A control experiment was done similar to each emulsion experiment, except that olive oil was omitted from the former.
2. If the PH of the emulsion mixture and the control mixture were brought to the value 8.0. before digestion began, than an emulsion of olive oil produced a slight increased amount of digestion and showed a higher PH throughout the experiment.
3. If the control mixture were brought to PH = 8.0 by the addition of alkali and an equal volume was added to the emulsion mixture then oil had no apparent effect on digestion, though the PH of the emulsion mixture became higher than the control during the first hour, and remained so throughout the experiment.

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