SUMMARY

1. The retention of Ca by rats receiving diets mainly consisting of wholemeal bread or of bread made from 72% extraction flour has been studied in metabolic experiments.

2. In one experiment *Creta praeparata* or bone meal were added to the 72% extraction flour to bring the Ca content of the diet to that of the wholemeal bread diet. In another, the two substances were added to the 72% extraction flour at a rate equivalent to $\frac{1}{2}$ lb. of CaCO_3 /280 lb. sack of flour, and the wholemeal flour was similarly supplemented to bring its Ca content to the same level.

3. In both instances Ca was less well retained from the wholemeal bread diets than from the diets containing bread made from lower extraction flour, and the evidence strongly suggests that this was due to the depressing effect of the phytic acid present in wholemeal bread. These results confirm the observations on man by McCance & Widdowson (1942a).

4. The wholemeal bread diets promoted better growth in rats than the 72% bread diets.

We are greatly indebted to Dr P. Halton of the Cereals Research Station of the Ministry of Food for the preparation of the breads used in this study and to Drs T. Moran, R. A. McCance and E. M. Widdowson for much help and advice in planning the experiment.

REFERENCES

- Brooke, R. O., Smith, A. H. & Smith, P. K. (1934). *J. biol. Chem.* **104**, 141.
- Chick, H. (1940). *Lancet*, **2**, 511.
- Chick, H. (1942). *Lancet*, **1**, 405.
- Copping, A. M. (1943). *Biochem. J.* **37**, 12.
- Ellis, M. & Mitchell, H. H. (1933). *Amer. J. Physiol.* **104**, 1.
- Giri, K. V. (1940). *Indian J. med. Res.* **28**, 101.
- Harrison, D. C. & Mellanby, E. (1939). *Biochem. J.* **33**, 1660.
- Henry, K. M., Houston, J., Kon, S. K., Powell, J., Carter, R. H. & Halton, P. (1941). *J. Dairy Res.* **12**, 184.
- Henry, K. M. & Kon, S. K. (1937). *Milk and Nutrition*, Part 1, p. 9. Reading: Nat. Inst. Res. Dairying.
- Henry, K. M. & Kon, S. K. (1939a). *Biochem. J.* **33**, 173.
- Henry, K. M. & Kon, S. K. (1939b). *Biochem. J.* **33**, 1652.
- Kon, S. K. (1943). *J. Dairy Res.* **13**, 216.
- Kon, S. K. & Henry, K. M. (1935). *Biochem. J.* **29**, 2051.
- Kramer, B. & Shear, M. J. (1928). *J. biol. Chem.* **79**, 147.
- Krieger, C. H., Bunkfeldt, R. & Steenbock, H. (1940a). *J. Nutrit.* **20**, 7.
- Krieger, C. H., Bunkfeldt, R. & Steenbock, H. (1940b). *J. Nutrit.* **20**, 15.
- Laskowski, M. (1937). *Biochem. Z.* **292**, 319.
- Lowe, J. T. & Steenbock, H. (1936). *Biochem. J.* **30**, 1991.
- McCance, R. A. & Widdowson, E. M. (1935). *Biochem. J.* **29**, 2694.
- McCance, R. A. & Widdowson, E. M. (1940). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 235.
- McCance, R. A. & Widdowson, E. M. (1942a). *J. Physiol.* **101**, 44.
- McCance, R. A. & Widdowson, E. M. (1942b). *J. Physiol.* **101**, 304.
- Marcy, L. F. (1944). *J. Nutrit.* **28**, 17.
- Moran, T. & Drummond, J. C. (1940). *Nature, Lond.*, **146**, 117.
- Patwardhan, V. N. (1937). *Biochem. J.* **31**, 560.
- Rogoziński, F. (1910). *Bull. int. Acad. Cracovie*, B, p. 260.
- Sherman, H. C. & Macleod, F. L. (1925). *J. biol. Chem.* **64**, 429.
- Sherman, H. C. & Quinn, E. J. (1926). *J. biol. Chem.* **67**, 667.
- 'Student' (1908). *Biometrika*, **6**, 1.
- 'Student' (1925). *Metron*, **5**, 105.
- Widdowson, E. M. (1941). *Nature, Lond.*, **148**, 219.
- Yudkin, J. (1943). *Biochem. J.* **37**, 543.

The Formation of Mono- and Di-glycerides during the Hydrolysis of Triglyceride by Pancreatic Lipase

By A. C. FRAZER AND H. G. SAMMONS, Sir Halley Stewart Research Fellows,
Pharmacology Department, University of Birmingham

(Received 1 January 1945)

It has been generally assumed that triglyceride is rapidly broken down by pancreatic lipase to fatty acid and glycerol. This is a necessary assumption if it is believed that triglyceride must be completely hydrolyzed to fatty acid and glycerol before it can be absorbed from the intestinal lumen (Verzar & McDougall, 1936; Bloor, 1943). It can be shown, however, that triglyceride and fatty acid behave differently both during and after absorption. Tri-

glyceride absorption is associated with globular dispersion of fat in the intestinal cell, with milkiness of the lacteals, with an increase of the particulate fat in the systemic blood and with subsequent deposition in the fat depots, whereas fatty acid absorption gives rise to a granular appearance in the intestinal cell, an increase of fatty material in the portal blood and deposition in the liver (Frazer, 1938, 1943a). It is generally agreed that trigly-

cerides are emulsified in the small intestine. An effective intestinal emulsifying system must fulfil four criteria. Emulsification must be spontaneous without violent agitation, the emulsion particle diameter should be less than 0.5μ , the emulsion must be stable for a period of at least 3 hr. and the system must be effective over a pH range of 6.0–8.0. The possible chemical factors in intestinal emulsification of fat have been studied in single, double and triple combination. The only system which satisfied the necessary conditions was the triple combination of monoglyceride/fatty acid/bile acid (Frazer, Schulman & Stewart, 1944). The object of this communication is to give evidence for the formation of mono- and di-glycerides (henceforth called lower glycerides) during pancreatic lipolysis both *in vitro* and *in vivo*. The possible significance of these lower glycerides in the absorption of fat from the intestine is discussed.

METHODS

Reagents. Olive oil (Pool; acetyl value 5, fatty acid content 0.3%) was used in these experiments to correlate them with previous biological studies.

Lipase A: Suspension (60 mg./ml.) of pancreatin (Parke Davis and Co.).

Lipase B: Suspension (60 mg./ml.) of dried pancreas (Evans, Sons, Lescher and Webb Ltd.).

Bile acids: Sodium tauroglycocholate (British Drug Houses Ltd.).

Buffer solutions: Appropriate mixtures of 0.1 M- KH_2PO_4 and 0.1 M- NaOH .

Standard procedure for hydrolysis and extraction of products. A mixture of buffer solution (5 ml.), aqueous lipase suspension (1 ml.), and bile acid solution (0.1 ml. of 0.25 M) was warmed to 37° ; olive oil (1 ml.), also at body temperature, was then added. The whole was incubated at 37° , the mixture being shaken by hand every 20 min. At the end of the experimental period glacial acetic acid (1 ml.) was added, and the mixture washed into a 250 ml. conical flask with a 50% ether-ethanol mixture (65 ml.). This mixture was heated to boiling to coagulate the proteins, allowed to cool, filtered, and the solvents removed *in vacuo* at 50° . To remove traces of acetic acid 50% ethanol (25 ml.) was now added and this was again removed *in vacuo*. Dehydration was effected by repeated treatment with ethanol, and the oily residue was extracted three times with ether and the extract filtered. The ether was removed *in vacuo* leaving an oil which was further examined; the non-oily residue was analyzed for glycerol. Control experiments were carried out using this technique with glycerol, glycerol and lipase, and glycerol and olive oil without lipase. Bile acids (0.5%); the probable concentration in intestinal contents) were normally added.

Analysis of hydrolysate. The acetyl value of the oily material was determined by the method described by West, Hoagland & Curtis (1934) using acetic anhydride in pyridine. The fatty acid value was determined by titration with sodium ethoxide. The glycerol was estimated by the method of Smith & Duke (1941).

Separation of lower glycerides. Various methods of separation of the lower glycerides from the glyceride residue

were attempted. Success was eventually achieved with phthalic anhydride, by a method modified from that of Coggan & Copenhagen (1939), who employed it for the separation of aliphatic alcohols. The method can be considered in three stages:

(1) Separation of glyceride residue. Digestion was allowed to proceed normally, and, at the conclusion of the experimental period, the mixture was made alkaline with 2N- Na_2CO_3 . The glyceride fraction was removed from the alkaline solution by continuous extraction with ether.

(2) Formation of phthalic acid derivative of lower glycerides. To the glyceride fraction an equimolecular proportion of phthalic anhydride was added together with anhydrous pyridine. The clear solution was heated at $105\text{--}110^\circ$ for 1 hr. After cooling, water (1 l.) was added, and the whole filtered through Kieselguhr under slightly reduced pressure till a clear filtrate was obtained. The Kieselguhr was extracted with ether which was dried over anhydrous Na_2SO_4 . When the solvent was removed, a clear yellow oil was obtained.

(3) Separation of phthalic acid derivatives. The yellow oil obtained above was exactly neutralized with 0.5N- Na_2CO_3 solution and the uncombined glyceride fraction removed by ether extraction. The remaining clear aqueous solution was then acidified with 2N-HCl and continuously extracted with ether, when a golden brown oil was finally obtained. The properties of this oil were examined and compared with an authentic phthalic derivative of monostearin. The acid value of the phthalate was estimated before and after hydrolysis, and the decomposition products were recovered quantitatively; these results were compared with the calculated theoretical values. The authentic specimen of monostearin phthalate was prepared from recrystallized monostearin (94% pure, m.p. $63\text{--}65^\circ$) by the method described above.

In vivo lipolysis. The animals used were young adult rats from our own breeding stock. To obtain the material from the lumen of the intestine, the animals were anaesthetized with ether, and the olive oil was injected intragastrically. For experimental periods of more than 6 hr. a ligature was applied to the ileo-caecal junction. At the end of the experiment the intestinal contents were removed and the material from 12 rats was pooled to provide sufficient for satisfactory analysis.

RESULTS

(1) The products of pancreatic lipolysis after periods of 2–48 hr.

In vitro lipolysis was carried out as described above for varying time intervals. The fatty acid content of the fatty material was estimated and the acetyl value of the glyceride residue was determined. The non-fatty residue was analyzed for free glycerol (Table 1).

(2) Destruction of glycerol during lipolysis

It has been claimed (Bull, 1937) that glycerol is destroyed in the course of lipolysis. Control experiments were therefore made to determine whether the lack of glycerol in the non-fatty residue was accounted for by non-liberation from the glycerides

Table 1. *The products of pancreatic lipolysis after periods of 2-48 hr.*

(The results for each hour represent the average of three experiments. Bile acid 0.5%, pH 7.0. A blank mixture without oil was analyzed simultaneously in each experiment as a check on the oxidizable substances present.)

Time (hr.)	0	2	3	4	5	9	12	24	48
Total substrate (mg.)	850	843	848	819	806	804	797	837	863
Total fatty acid (mg.)	2.5	201	251	261	270	308	322	432	486
% lipolysis	0.3	24.2	29.7	31.6	33.5	38.4	40.8	51.1	56.2
Acetyl value (mg. KOH to neutralize acetic acid liberated on saponification of 1 g. of acetylated product)	5.0	47.2	58.0	60.8	64.0	67.7	74.5	77.6	66.7
% glyceride residue as monoglycerides* (calc. from fatty acid value)	—	19.9	26.6	28.8	31.7	39.4	43.3	68.6	94.0
% glyceride residue as monoglycerides* (calc. from acetyl value)	—	19.9	26.6	28.8	31.0	35.6	40.6	53.6	55.0
Free glycerol (mg.)	—	0.0	0.0	0.0	1.8	4.2	6.3	12.0	6.0
Expected glycerol (mg.) if hydrolysis complete	—	21.9	27.7	29.0	29.7	33.5	35.3	47.8	53.0

* The lower glycerides are calculated as monoglycerides in this and subsequent tables, although in most cases they consist of a mixture of di- and monoglycerides.

or by its subsequent destruction. It will be seen from Table 2 that glycerol shows no significant destruction during the first 5 hr. of lipolysis and destruction is not marked until after 24 hr.

Table 2. *Destruction of glycerol during lipolysis at pH 7.0*

Time in hr.	Composition of substrate mixture				
	Glycerol only	Glycerol with oil	Lipase, bile acid (0.5%), glycerol		
	0	0	2	24	48
Glycerol taken (mg.)	20.3	57.2	20.3	20.3	20.3
Glycerol found (mg.)	20.4	54.5	19.7	17.3	2.9

(3) Relationship of potency of lipase to lower glyceride formation

A. *Effect of varying pH.* The effect of pH, which is known to influence lipolytic activity, was tested upon the formation of lower glycerides. It will be seen from Table 3 that the amount of lower glycerides formed in relation to fatty acid liberated is not affected over the pH range of 6-10.5.

B. *Effect of varying bile acid concentration.* Table 4 shows the results obtained. Increase of bile acids from 0.5% upwards did not result in any decrease of lower glyceride formation.

C. *Effect of varying enzyme/substrate ratio.* The third possible factor which might account for partial instead of complete hydrolysis of triglyceride was

Table 3. *Effect of varying pH on lipolysis*

(Substrate mixture contained 0.5% bile acid.)

pH	6.0	7.0	7.5	8.0	10.5
Time (min.)	120	120	120	120	120
Total substrate (mg.)	869	813	772	841	820
Total fatty acid (mg.)	184	201	211	221	185
% lipolysis	22.4	24.2	27.4	26.3	22.6
Acetyl value	44.3	47.2	54.2	52.1	44.8
% glyceride residue as monoglycerides (calc. from fatty acid value)	18.1	19.9	23.7	22.4	18.4
% glyceride residue as monoglycerides (calc. from acetyl value)	18.1	19.9	23.7	22.4	18.4
Free glycerol (mg.)	0.0	0.0	0.0	0.0	0.0
Expected glycerol (mg.) if complete hydrolysis	20.0	21.9	23.0	24.0	20.0

Table 4. *Effect of varying bile acid concentration at pH 7.5 on lipolysis*

	Bile acids added as % of total solution						
	...	0	0.2	0.5	1	2	4
Time (min.)		120	120	120	120	120	120
Total substrate (mg.)		771	717	772	753	723	749
Total fatty acid (mg.)		21.8	150	211	222	209	251
% lipolysis		2.8	20.9	27.4	29.5	28.9	33.5
Acetyl value		5.6	41.5	54.2	58.5	57.4	66.5
% glyceride residue as monoglycerides (calc. from fatty acid value)		1.9	16.6	23.7	26.4	25.6	31.8
% glyceride residue as monoglycerides (calc. from acetyl value)		1.9	16.6	23.7	26.4	25.6	31.8
Free glycerol (mg.)		0.0	0.0	0.0	0.0	—	—
Expected glycerol (mg.) if complete hydrolysis		2.38	16.3	23.0	24.1	22.7	27.3

* Results not reliable owing to frothing during concentration.

the amount of enzyme present. This was increased in two ways—by addition of more lipase solution, and by increasing the amount of pancreatic extract in the lipase solution. The results are shown in Table 5. It will be seen that increases up to fourfold did not result in any diminution in the proportion of lower glycerides formed.

(4) *Separation of lower glycerides from glyceride residue*

The glyceride residue was fractionated by the method described above. The results are shown in Table 6.

Table 5. *Effect of varying enzyme/substrate ratio on lipolysis*

(i) Effect of addition of more lipase (60 mg. pancreatin/ml.) at pH 7.0

	Lipase suspension added (ml.)			
	1	2	4	8
Time (min.)	120	120	120	120
Total substrate (mg.)	813	794	811	831
Total fatty acid (mg.)	201	194	213	194
% lipolysis	24.2	24.4	26.3	23.4
Acetyl value	47.2	48.5	52.2	46.4
% glyceride residue as monoglycerides (calc. from fatty acid value)	19.9	20.4	21.5	19.2
% glyceride residue as monoglycerides (calc. from acetyl value)	19.9	20.4	21.5	19.2
Free glycerol (mg.)	0.0	0.0	0.0	0.0
Expected glycerol (mg.) if complete hydrolysis	21.9	21.1	23.2	21.1

(ii) Effect of increasing the concentration of pancreatin at pH 7.5

	Concentration of lipase (pancreatin (mg.) contained in 1 ml. of suspension)				
	60	120	120	240	240
Time (min.)	120	120	120	120	120
Substrate (mg.)	772	804	825	812	797
Total fatty acid (mg.)	211	204	233	213	211
% lipolysis	27.4	25.4	28.2	26.2	26.5
Acetyl value	54.2	50.1	56.0	52.1	52.6
% glyceride residue as monoglycerides (calc. from fatty acid value)	23.7	21.4	24.8	22.5	22.7
% glyceride residue as monoglycerides (calc. from acetyl value)	23.7	21.4	24.8	22.5	22.7
Free glycerol (mg.)	0.0	0.0	0.0	0.0	0.0
Expected glycerol (mg.) if complete hydrolysis	23.0	22.2	25.3	23.2	23.0

Table 6. *Separation of lower glycerides from glyceride residue*

	Properties of lower glycerides				
	Authentic monostearin diphthalate		Specimen I	Diolein mono-phthalate (calc.)	Specimen II
	Actual	Calc.			
Acid value (mg. KOH equiv. to 1 g.):					
(a) Before hydrolysis	172	171	148	73	77
(b) After hydrolysis	399	428	346	292	297
Decomposition of 1 g.:					
(a) Phthalic acid (mg.)	448	508	404	216	213
(b) Fatty acid (mg.)	448	434	591	734	679
(c) Glycerol (mg.)	112	141	—	120	133

Solubility. All these phthalic acid derivatives are soluble in ether, chloroform, benzene, acetone and ethanol, but insoluble in water.

Melting-point. Monostearin diphthalate is an oil at room temperature.

Authentic preparation. Condensation of monostearin with phthalic anhydride did not give a quantitative yield of diphthalate, but this was separated by solution of its sodium salt in cold water.

Specimen I is a mixture of approximately two-thirds monoglyceride diphthalate and one-third diglyceride mono-phthalate.

Specimen II is almost pure diglyceride monophthalate.

(5) *Formation of lower glycerides in vivo*

Material obtained from 12 rats was analyzed. Each animal was given 2 ml. olive oil intragastrically and killed 16 hr. later. The intestinal contents were found to contain 384 mg. of fatty acid, representing 30% lipolysis. The acetyl value of the glyceride residue was 70, equivalent to 31.8% of monoglyceride.

DISCUSSION

There are at least four sources of lipase in the alimentary tract: gastric, pancreatic and intestinal secretions, and lipoclastic bacteria. Of these, the most important for absorption of fat is the pancreatic lipase. Pancreatic secretion is brought about by two mechanisms, a reflex flow initially which is rich in ferments, and a more prolonged hormonal flow with a poor enzyme content (J. Mellanby, 1925). With moderate amounts of fat the peak lipaemia in the systemic blood occurs 2½–3 hr. after ingestion (Frazer & Stewart, 1937), and the blood fat has returned to the normal level within 4½ hr. Larger amounts of fat may cause a delay in the maximum lipaemia for periods up to 6 hr. after ingestion, mainly due to increase in stomach emptying time. Therefore, the first 5 hr. of pancreatic lipolysis is the important period biologically.

It is clear from the experiments described that the products of pancreatic lipolysis during the first 5 hr. are fatty acid and lower glycerides. The increase in the acetyl value of the glyceride fraction and the absence of free glycerol in the non-fatty residue can hardly be interpreted otherwise. This conclusion is further strengthened by the separation of the phthalic acid derivatives of the lower glycerides, the properties of which compare reasonably well with those of an authentic preparation. The correlation between the actual and expected figures in this experiment is reasonably close when it is considered that the preparations studied have been derived from a natural mixed triglyceride. The formation of lower glycerides has been reported previously for hydrolysis by acid and by ricinus lipase (Armstrong & Gosney, 1914; Grün & Corelli, 1912), but so far as we are aware it has not been previously reported during pancreatic lipolysis and certainly has not been stressed. The absence of free glycerol has been explained (Bull, 1937) as being due to glycerol destruction, but this is not a significant factor for the first 5 hr.

It seemed possible that the formation of lower glycerides might be an expression of low lipolytic potency in our pancreatic preparations. But no significant differences in the proportion of lower glycerides formed or in the liberation of glycerol during the first 5 hr. of hydrolysis could be demonstrated when different sources of lipase were used, when the pH was varied between 6 and 10.5, when

the bile concentration was altered from 0.2 to 4%, or when the lipase present was increased up to fourfold.

When the amount of lipase was increased, there was no marked rise in the percentage lipolysis. This is in accord with the findings of Willstätter, Waldschmidt-Leitz & Memmen (1922), who showed that the addition of lipase beyond a certain point causes no further enhancement of hydrolysis. The amount of lipase used in our experiments is well above this limiting value. The inability to cause increased lipolysis *in vitro* appears to be in contradiction to the observation made in human subjects and animals that the addition of lipase after ingestion of fat causes a depression of the post-absorptive systemic lipaemia (Frazer, 1943b). The explanation of this apparent anomaly may be that the lipase which is normally secreted into the intestine is below the limiting value described by Willstätter *et al.* (1922). Alternatively, the difference may be due to the time relationships of the addition of lipase to the incidence of emulsification, a possible explanation of the failure of Balls, Matlack & Tucker (1937) to demonstrate monostearin in their investigation of tristearin hydrolysis. These, and other possibilities, are under investigation.

It has been frequently suggested that lipolysis may occur more rapidly in the intestine than *in vitro* because the end-products are removed. It is doubtful, however, whether the Law of Mass Action is applicable in this way to the hydrolysis of fat suspended in an aqueous medium, and there is little doubt that the slowing up of hydrolysis *in vitro* is not due to the establishment of a simple equilibrium due to the accumulation of end-products. However, to ensure that complete hydrolysis does not occur when lipase is acting in the intestinal lumen, the intestinal contents of a group of animals were examined. The content of lower glycerides in this material was comparable to that found in our other experiments.

The formation of lower glycerides during pancreatic lipolysis is probably essential for the emulsification of fat in the small intestine. Up to the present time no satisfactory explanation of this phenomenon has been put forward. It has been attributed to the bile acids, but it can be shown that these substances, if freed from soaps, do not lower the interfacial tension between oil and water sufficiently to allow more than crude emulsification, with particles 2–10 μ in diameter and of low stability. Another emulsifier suggested is soap, which will lower the interfacial tension to suitable levels provided that the reaction is alkaline. There is no doubt, however, that the pH of the contents of the small intestine may range from pH 6.3 to 8.0, and the usual reaction is pH 6.5 (Kostyal, 1926; Robinson, 1935). Soap is quite ineffective as an emulsi-

fying agent below pH 7.5, especially in the presence of small quantities of calcium salts. The triple combination of monoglyceride/fatty acid/bile acid is an effective system under the conditions found in the intestine (Frazer *et al.* 1944). If this is the normal emulsifying system, then one of the functions of lipolysis is to provide two of the three essential constituents for emulsification.

Is fine emulsification provided to enable particulate absorption to occur, or is it simply a preliminary stage to more extensive lipolysis? Since finely dispersed paraffin, upon which lipase can make no impression, is rapidly absorbed from the small intestine (Frazer *et al.* 1944), it seems reasonable to suppose that finely dispersed triglyceride fat can be similarly absorbed without further hydrolysis. Furthermore, since no free glycerol is demonstrable during the first 5 hr. of pancreatic lipolysis, and 95% of ingested fat disappears from the intestinal lumen within this time, the substances absorbed can only be fatty acids and tri-, di- or monoglycerides. The essential point of the Lipolytic Hypothesis of fat absorption is the changing of glycerides into water-soluble components with the assistance of the hydrotropic action of the bile acids. The lower glycerides of the long-chain fatty acids are insoluble in water, and in the absence of any evidence of hydrotropes which act on monoglycerides, it must be assumed that these substances are absorbed in an insoluble form; probably by the same mechanism as triglycerides. It is suggested that the Partition Hypothesis of fat absorption provides a more satisfactory explanation of the available facts so far as the absorption of olive oil is concerned.

Fine emulsions of oil in water may flocculate in the presence of protein. This phenomenon can be shown to be dependent upon the nature of the film at the oil/water interface, the type of protein and the pH of the continuous phase (Elkes, Frazer, Schulman & Stewart, 1945). The flocculation reactions of the emulsion particle in the intestinal lumen are those of a negatively charged emulsion, and these globules are unaffected by *Cl. welchii* type A toxin lecithinase (Macfarlane & Knight, 1941). It can also be shown by analyses that there is no phospholipid in this fatty material. On the other hand, the fat particles found in the blood at the height of fat absorption give flocculation reactions similar to those obtained with a phospholipid

stabilized emulsion, and *Cl. welchii* lecithinase causes a loss of stability of these oil globules (Elkes & Frazer, 1944). It is suggested that the fat particle is coated with phospholipid in the course of absorption, probably in the intestinal cell. It is likely that this phospholipid is formed at the oil/water interface, and it is tentatively suggested that the lower glyceride which is concentrated at this interface may form the basis for phospholipid synthesis.

SUMMARY

1. During the first 5 hr. of pancreatic lipolysis of olive oil no free glycerol can be demonstrated and the acetyl value of the glyceride fraction rises from 5 to 64.

2. No significant glycerol destruction can be demonstrated during the first 5 hr. of pancreatic lipolysis.

3. The formation of lower glycerides is not a result of low potency of the lipase.

4. The lower glycerides can be separated from the glyceride residue by condensation with phthalic anhydride.

5. Material recovered from the intestines of a group of 12 rats shows a proportion of lower glycerides comparable to the *in vitro* experiments.

6. It is concluded that fatty acids and di- and monoglycerides are the only products of hydrolysis of olive oil by pancreatic lipase during the first 5 hr. of digestion.

7. An emulsifying system for fat which is effective under the conditions prevailing in the intestine is the triple combination fatty acid/bile acid/lower glyceride. One of the functions of lipolysis would appear to be the formation of two of the three essential factors for emulsification.

8. The partial hydrolysis of glycerides, demonstrable during the first 5 hr. of lipolysis, is in accord with the Partition Hypothesis of fat absorption, but not with the Lipolytic Hypothesis.

9. It is suggested that lower glycerides may form the basis for the formation of phospholipid at the oil/water interface in the intestinal cell.

We wish to express our indebtedness to Prof. H. P. Gilding and Dr W. V. Thorpe for facilities provided in the Biochemistry Department here, to Prof. T. P. Hilditch, F.R.S., for helpful criticism and advice, and to Mr Gormley, of John Wyeth and Co., for a supply of monoglyceride. We also wish to thank the Sir Halley Stewart Trust for their financial assistance.

REFERENCES

- Armstrong, H. E. & Gosney, H. W. (1914). *Proc. roy. Soc. B*, **88**, 176.
Balls, A. K., Matlack, M. B. & Tucker, I. W. (1937). *J. biol. Chem.* **122**, 125.
Bloor, W. R. (1943). *Biochemistry of the Fatty Acids*. New York: Reinhold Publishing Corporation.
Bull, H. B. (1937). *Biochemistry of the Lipids*, p. 51. New York: J. Wiley & Sons Inc.
Coggan, J. F. & Copenhaver, J. E. (1939). *J. Amer. chem. Soc.* **61**, 2909.
Elkes, J. J. & Frazer, A. C. (1944). *J. Physiol.* **102**, 24P.

- Elkes, J. J., Frazer, A. C., Schulman, J. H. & Stewart, H. C. (1945). In the Press.
- Frazer, A. C. (1938). *Analyst*, **63**, 308.
- Frazer, A. C. (1943*a*). *J. Physiol.* **102**, 306.
- Frazer, A. C. (1943*b*). *J. Physiol.* **102**, 329.
- Frazer, A. C., Schulman, J. H. & Stewart, H. C. (1944). *J. Physiol.* **103**, 306.
- Frazer, A. C. & Stewart, H. C. (1937). *J. Physiol.* **90**, 18.
- Grün, A. & Corelli, O. (1912). *J. chem. Soc.* p. 409.
- Kostyal, L. (1926). *Mag. orv. Arch.* **27**, 276.
- Macfarlane, M. G. & Knight, B. C. J. G. (1941). *Biochem. J.* **35**, 884.
- Mellanby, J. (1925). *J. Physiol.* **60**, 85.
- Robinson, C. S. (1935). *J. biol. Chem.* **108**, 403.
- Smith, G. F. & Duke, F. R. (1941). *Industr. Engng Chem. (Anal. ed.)*, **13**, 558.
- Verzar, F. & McDougall, E. J. (1936). *Absorption from the Intestine*. London: Longmans, Green and Co.
- West, E. S., Hoagland, C. L. & Curtis, G. H. (1934). *J. biol. Chem.* **104**, 627.
- Willstätter, R., Waldschmidt-Leitz, E. & Memmen, F. (1922). *Hoppe-Seyl. Z.* **125**, 93.

The Thermal Destruction of Vitamin B₁

1. THE INFLUENCE OF BUFFER SALTS ON THE RATE OF DESTRUCTION OF ANEURIN AT 100°

By K. T. H. FARRER, *Research Division, Kraft Walker Cheese Co. Proprietary Ltd., Melbourne, Australia*

(Received 3 January 1945)

The rate of destruction of aneurin in boiling solutions at various pH values has been described by Farrer (1941*a*). It was realized that the work done was, in effect, the study of a first order reaction, but a formal mass-action study was not published. Traill (1941) subsequently pointed out that if $\log k$ (where k is the velocity coefficient) is plotted against pH (also a logarithmic function), the curve shown in Fig. 1 results. The relation is linear until pH 7 is reached, after which the curve rises more steeply. Further work on this problem was not possible until recently. A reference to some work by Beadle, Greenwood & Kraybill (1943), who claim that aneurin is destroyed at different rates in different buffer solutions, focused attention on the fact that in the work previously published two buffer solutions were used, the McIlvaine phosphate-citric acid buffer for pH 3, 4, 5 and 6, and the Kolthoff phosphate-borate buffer for pH 7, 8 and 9. Booth (1943), too, using phosphate and phthalate buffers, noted rates of destruction smaller than those described earlier (Farrer, 1941*a*), and suggested that the buffer used in that work contained some impurity which catalyzed the destruction of aneurin. This was not so, and it was immediately realized that the change in slope of the curve shown in Fig. 1 could be due to the occurrence of two different straight lines, one for each buffer solution. This explanation of the change of slope has been proved correct by further experimental work, and it has been found that other buffer solutions also show a linear relation between pH and $\log k$. Furthermore, it has been found that the slope of the curve obtained by plotting pH

against $\log k$ varies with the buffer solution used and with the predominating ions present.

Fig. 1. pH/ $\log k$ curve for the pH values studied earlier (Farrer, 1941*a*).

EXPERIMENTAL

The method of attack was the same as that previously reported. 25 ml. of aneurin stock solution were added to 200 ml. of the buffer solution and the pH precisely determined. The solution was then boiled under reflux, and samples were withdrawn at regular intervals, cooled as rapidly as possible, and the aneurin content determined by the Jansen thiochrome method.

Aneurin stock solution. Several different solutions were used throughout this work, but all were made by dissolving about 10 mg. of pure vitamin B₁ (obtained from Bayer Pharma Proprietary Ltd., Sydney) in 250 ml. of water distilled in glass. As previously reported, two or three drops of HCl added to these stock solutions render them stable for at least 3 months.