7. SDS (0.001M) inhibits succinic dehydrogenase to a less extent than it does the whole succinic oxidase system, possibly owing to its combination with MB. SDS has no effect on cytochrome oxidase.

8. SDS (0.0002M) strongly inhibits invertase at pH 4.0. This inhibition can be completely abolished by 0.067M fructose but not by glucose.

9. SDS has little effect on the hydrolysis of casein by trypsin, but it strongly stimulates digestion of native serum proteins and to a less degree that of the denatured proteins.

The author wishes to express his thanks to Prof. A. Wormald for advice and interest in this work. Thanks are also tendered to Mr S. Ellingworth of Messrs Imperial Chemical Industries Ltd. for a supply of Antrypol amine.

REFERENCES

- Cooper, J. A. & Bull, H. B. (1944). *Abstracts of 107th Meeting of Amer. chem. Soc.*, p. 31B. Cleveland, Ohio.
- Glick, D. & King, C. G. (1932). *J. biol. Chem.* **97**, 675.
- Guimaraes, J. L. & Lourie, E. M. (1951). *Brit. J. Pharmacol.* **6**, 514.
- Harrison, G. A. (1947). *Chemical Methods in Clinical Medicine*, 3rd ed. p. 475. London: Churchill.
- Hartley, G. S. (1936). *Aqueous Solutions of Paraffin Chain Salts*. Paris: Hermann et Cie.
- Haurowitz, F., Tunca, M., Schwerin, P. & Goksu, V. (1945). *J. biol. Chem.* **157**, 621.
- Holwerda, K. (1938). *Biochem. Z.* **296**, 1.
- Krebs, H. A. (1935). *Biochem. J.* **29**, 1620.
- Lundgren, H. P. (1945). *Text. Res. (J.)*, **15**, 335.
- Marron, T. U. & Moreland, F. B. (1939). *Enzymologia*, **6**, 225.
- Putnam, F. W. (1948). *Advanc. Protein Chem.* **4**, 79.
- Putnam, F. W. & Neurath, H. (1944). *J. Amer. chem. Soc.* **66**, 692.
- Quastel, J. H. & Yates, E. D. (1936). *Enzymologia*, **1**, 60.
- Rabinovitz, M. & Boyer, P. D. (1950). *J. biol. Chem.* **183**, 111.
- Ralston, A. W. (1946). *Ann. N.Y. Acad. Sci.* **46**, 351.
- Slater, E. C. (1949). *Biochem. J.* **45**, 14.
- Steinhardt, J. (1941). *J. biol. Chem.* **141**, 995.
- Sumner, J. B. & Somers, G. F. (1947). *Chemistry and Methods of Enzymes*, 2nd ed. p. 69. New York: Academic Press, Inc.
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1949). *Manometric Techniques and Related Methods for the Study of Tissue Metabolism*, 2nd ed. Burgess: Minneapolis.
- Van Antwerpen, F. J. (1943). *Industr. Engng Chem. (Industr.)*, **35**, 126.
- Weinstein, S. S. & Wynne, A. M. (1936). *J. biol. Chem.* **112**, 641.
- Wills, E. D. (1952a). *Biochem. J.* **50**, 421.
- Wills, E. D. (1952b). *2nd Int. Congr. Biochem. Abstr.* p. 237.
- Wills, E. D. (1953). *Biochem. J.* **53**, xx.
- Wills, E. D. & Wormald, A. (1950a). *Nature, Lond.*, **165**, 813.
- Wills, E. D. & Wormald, A. (1950b). *Biochem. J.* **47**, 158.
- Willstätter, R. & Memmen, F. (1924). *Z. phys. Chem.* **133**, 229.
- Willstätter, R., Waldschmidt-Leitz, E. & Memmen, F. (1923). *Z. phys. Chem.* **125**, 93.

Dietary Fat and Body Fat: the Composition of the Back Fats of Pigs Fed on a Diet Rich in Cod-liver Oil and Lard

BY G. A. GARTON AND W. R. H. DUNCAN
Rowett Research Institute, Bucksburn, Aberdeenshire

(Received 9 December 1953)

In a recent study (Garton, Hilditch & Meara, 1952) of the depot fats of a pig fed on a diet rich in whale oil it was found that apparently unchanged whale-oil glycerides were present in the depots. However, since the whale oil was ingested in very large amounts and represented practically all the fat in the diet, the depot glycerides which resembled whale oil would have been substantially the same whether they had been hydrolysed and resynthesized during passage from the intestine to the lymphatics or whether they had been absorbed intact. That the pig is capable of depositing dietary fatty acids in its fat depots is well known (for a review see Hilditch, 1947, pp. 99–101), though it is not known to what extent dietary triglycerides are absorbed as such and deposited in the fat depots.

The present study was undertaken to obtain evidence in this direction by feeding a diet containing a mixture of lard and cod-liver oil to two young pigs and then studying the composition of the resultant depot fats. If intestinal hydrolysis occurred to any appreciable extent, followed by resynthesis in the intestinal wall or elsewhere, the component fatty acids characteristic of cod-liver oil (especially the polyethenoid C₂₀–C₂₂ acids) and of lard would mix and result in the formation of depot glycerides which would not be separable by low-temperature crystallization into groups of glycerides resembling lard and cod-liver oil, respectively. As described below, such a separation was, however, effected, although the cod-liver oil had undergone oxidative changes, and it seems therefore that

intestinal hydrolysis of the ingested fats with subsequent resynthesis of 'new' glycerides had not taken place to any appreciable extent.

METHODS AND RESULTS

Animals and diet

Two normal Large White $\times$ Wessex litter-mate piglets (1 gilt and 1 hog) were fed from weaning at 8 weeks on a diet containing commercial lard and cod-liver oil as a mixture of equal parts by weight; the analytical values of the two fats are given in Table 1.

The amount of fat mixture was gradually increased until at the beginning of the seventh week of the experiment it comprised 50% by weight of the diet. Full details of the diet, feeding scale, management of the animals and their weight gains have been given by Garton & Naftalin (1953).

No supplementary antioxidant was included in the diet since Walley (1952), at the Animal Research Station, School of Agriculture, Cambridge, found that pigs could be raised to bacon weight (200 lb.) on a diet containing 50% crude whale oil without added vitamin E. The mean unsaturation of the C_{20} and C_{22} polyethenoid fatty acids of cod-liver oil and whale oil is of the same order (see Hilditch, 1947), and it was calculated that the amounts of these acids in a diet containing 25% of cod-liver oil would be roughly the same as that furnished by 50% of whale oil. However, during the seventh week of the present experiment, the animals suddenly lost their appetites and became oedematous and unsteady in their gait. They were killed at the end of the seventh week and examined *post mortem*. The morbid anatomical findings, which resembled the exudative condition induced in chickens fed on high-fat diets deficient in vitamin E (Dam, 1944*a, b*), have already been described in detail (Garton & Naftalin, 1953).

Extraction of back fats and their characteristics

As much as possible of the inner- and outer-back adipose tissue was removed from each animal; there was an almost complete absence of perinephric fat. The tissues, which

were stored at 2° overnight, were cut into small pieces and exhaustively extracted with boiling acetone. The acetone-soluble material was extracted with light petroleum (b.p. 40–60°) and the lipid material from each tissue was separately recovered and found to have the analytical characteristics shown in Table 2.

All four fats were dark brown and semi-solid at room temperature; they had a green fluorescence in daylight and an odour of cod-liver oil.

Separation of the component glycerides of the back fats of the hog

The characteristics given in Table 2 showed that all the fats were considerably peroxidized, which would probably render the determination of their polyethenoid acid content very difficult. Nevertheless, it was decided to attempt a separation, by low-temperature crystallization, of the glycerides of the back fats of one animal. Preliminary experiments showed that mixtures of cod-liver oil and lard could be effectively resolved by low-temperature crystallization. Mixtures of iodine value 80–90 were separated almost completely, as judged by analytical results (iodine value and saponification equivalent), into their two components by successive crystallizations at –40° of the lard glycerides from a 10% solution of the mixture in acetone.

A quantity of each of the inner- and outer-back fats of the hog was therefore crystallized from 10% solution in acetone at –40°, the deposited (more saturated) glyceride being recrystallized under the same conditions and the procedure repeated until only a small amount of glycerides remained in solution. In this way the fractions shown in Table 3 were obtained.

The respective portions left in solution from five crystallizations were pooled and so from each fat two groups of glycerides were obtained, the characteristics of which are given in Table 4.

Separation of the component fatty acids of the back fats of the hog

Each group of glycerides was saponified and the fatty acids removed by extraction with peroxide-free ether. The acids were then crystallized for 4–5 hr. from 10% solution in acetone or peroxide-free ether. The results of the solvent separations are shown in Table 5.

Fractionation of the methyl esters of the fatty acids

Each group of fatty acids derived by low-temperature crystallization was converted into methyl esters by refluxing with methanol containing 1% H_2SO_4 . The esters were

Table 1. Analytical characteristics of lard and cod-liver oil as fed to pigs

	Lard	Cod-liver oil
Iodine value	60.4	185.1
Saponification equivalent	284.5	301.3
Peroxide value (ml. 0.002N- $Na_2S_2O_3$ /g.)	1.0	11.7
Free fatty acid (as oleic, %)	0.25	0.04
Unsaponifiable matter (%)	0.80	1.80

Table 2. Analytical characteristics of inner- and outer-back fats

	Source of fat			
	Hog		Gilt	
	Inner back (348 g.)	Outer back (482 g.)	Inner back (239 g.)	Outer back (333 g.)
Iodine value	81.8	86.8	84.5	85.5
Saponification equivalent	277.8	282.0	278.7	278.9
Peroxide value*	25.3	34.1	55.0	28.3
Free fatty acid (as oleic, %)	1.2	1.1	1.4	1.1
Unsaponifiable matter (%)	0.84	0.40	0.44	0.44

* Determined immediately after removal of extraction solvents.

washed with 1% (w/v) Na_2CO_3 , dried and fractionally distilled at approximately 0.1 mm. pressure through an electrically heated column. The methods employed in the distillation and for the analytical (including spectrophotometric) examination of the ester fractions were those described by Gupta & Hilditch (1951). The composition of each ester fraction was then calculated according to the methods given by Hilditch (1947, pp. 498–510) and Gupta & Hilditch (1951), and therefrom the percentage composition (by weight) of each group of acids from each fat was derived.

(a) *Outer-back fat.* The distillation residues of the methyl esters of the acids of group *D* (Table 5) amounted to 15% and showed a fall from an iodine value of 286.4 (last distilled ester fraction) to 139.7 for the residual esters freed from

unsaponifiable matter. It was therefore apparent that destruction of polyethenoid acids had taken place to a very considerable extent and that calculation of the fatty acid composition of this fraction (and hence of the whole group of acetone-soluble glycerides) was not possible. Accordingly, the fatty acids of groups *A*, *B* and *C* of this fat were not submitted to ester fractionation.

(b) *Inner-back fat.* It was possible in this case to compute the fatty acid composition of each ester fraction and hence of each group of glycerides, the composition of which is given in Table 6. The composition of the residual esters of the fatty acids of group *E* (Table 5), after removal of unsaponifiable matter, was calculated as described by Hilditch (1947, p. 509), thus showing acids of somewhat higher unsaturation than were present in the fat as analysed.

Table 3. *Segregation of back-fat glycerides of hog by successive crystallization from 10% solution in acetone at -40°*

The weights of material refer to the fat remaining in solution, except for the last figure in each 'weight' column, which refers to the insoluble glycerides.

	Outer-back fat (245.0 g.)		Inner-back fat (227.6 g.)	
	Weight (g.)	Iodine value	Weight (g.)	Iodine value
First crystallization	46.0	150.7	41.9	133.0
Second crystallization	19.3	126.7	15.7	116.6
Third crystallization	5.2	111.1	6.6	108.8
Fourth crystallization	4.3	103.0	5.3	99.6
Fifth crystallization	4.0	99.1	4.5	98.5
Glycerides deposited (fifth crystallization)	166.2	64.0	153.6	60.3

Table 4. *Groups of glycerides separated from hog back fats*

	Outer-back fat			Inner-back fat		
	Weight		Iodine value	Weight		Iodine value
	(g.)	(% of total)		(g.)	(% of total)	
Glycerides insoluble in acetone at -40°	166.2	67.8	64.0	153.6	67.5	60.3
Glycerides soluble in acetone at -40°	78.8	32.2	137.0	74.0	32.5	126.5

Table 5. *Low-temperature crystallization of the mixed acids from each group of hog back-fat glycerides*

Fraction	Conditions	Weight		Iodine value
		(g.)	(% of total)	
Outer-back fat				
(1) Acids from glycerides insoluble in acetone at -40°				
A	Insoluble in ether at -35°	67.4	46.8	24.6
B	Soluble in ether at -35°	76.5	53.2	105.1
(2) Acids from glycerides soluble in acetone at -40°				
C	Insoluble in acetone at -40°	21.7	31.7	35.3
D	Soluble in acetone at -40°	46.7	68.3	169.0
Inner-back fat				
(1) Acids from glycerides insoluble in acetone at -40°				
A	Insoluble in ether at -30°	43.1	31.6	3.4
B	Soluble in ether at -30°	38.3	28.0	61.0
C	Soluble in acetone at -35°	55.2	40.4	109.5
(2) Acids from glycerides soluble in acetone at -40°				
D	Insoluble in acetone at -45°	30.2	45.4	70.9
E	Soluble in acetone at -45°	36.3	54.6	179.5

Table 6. *Component acids of solvent-separated fractions from each group of glycerides of the hog inner-back fat*Separation into fractions *A*, *B*, *C*, *D* and *E* is shown in Table 5.Acids from glycerides insoluble in acetone at -40°

Acid	Fraction			Fatty acids in whole group of glycerides (excluding unsaponifiable matter)	
	<i>A</i> (%)*	<i>B</i> (%)*	<i>C</i> (%)*	(%, w/w)	(%, by mol.)
Myristic	—	0.25	0.29	0.5	0.6
Palmitic	22.99	7.57	1.58	32.2	34.3
Stearic	7.41	1.59	—	9.0	8.6
Arachidic	0.18	—	—	0.2	0.2
Unsaturated C_{14}	—	0.06	0.45	0.5	0.6
Unsaturated C_{16}	0.05	1.20	2.70	4.0	4.2
Unsaturated C_{18}	0.97	17.23	30.18	48.4	46.9
Unsaturated C_{20}	—	—	5.20	5.2	4.6
Unsaponifiable	—	0.10	—	—	—

Mean unsaturation† of:

C_{14} acids	—	-2.0	-2.0	-2.0
C_{16} acids	-2.0	-2.0	-2.4	-2.3
C_{18} acids	-2.0	-2.0	-2.6	-2.4
C_{20} acids	—	—	-4.0	-4.0

Acids from glycerides soluble in acetone at -40°

Acid	Fraction		Fatty acids in whole group of glycerides (excluding unsaponifiable matter)	
	<i>D</i> (%)*	<i>E</i> (%)*	(%, w/w)	(%, by mol.)
Myristic	—	0.38	0.4	0.5
Palmitic	12.94	1.80	14.9	16.6
Stearic	2.36	—	2.4	2.4
Unsaturated C_{14}	—	1.34	1.3	1.6
Unsaturated C_{16}	9.08	8.60	17.9	19.0
Unsaturated C_{18}	19.02	24.42	43.9	42.3
Unsaturated C_{20}	2.00	12.51	14.7	13.7
Unsaturated C_{22}	—	4.48	4.5	3.9
Unsaponifiable	—	1.07	—	—

Mean unsaturation† of:

C_{14} acids	—	-2.0	-2.0
C_{16} acids	-2.0	-2.9	-2.4
C_{18} acids	-2.3	-3.5	-3.0
C_{20} acids	-2.0	-7.0	-6.3
C_{22} acids	—	-10.3	-10.3

* Component acids as % (w/w) of each solvent-separated fraction.

† Mean unsaturation expressed as number of H atoms required to convert 1 mol. of acid into the saturated state.

Table 7. *Separation of mixed fatty acids of lard and cod-liver oil by low-temperature crystallization*

Solubility characteristics of fraction	Weight		Iodine value
	(g.)	(% of total)	
Lard			
<i>A</i> Insoluble in ether at -30°	93.1	44.3	9.7
<i>B</i> Soluble in ether at -30°	78.7	37.4	82.1
<i>C</i> Soluble in acetone at -60°	38.5	18.3	145.0
Cod-liver oil			
<i>A</i> Insoluble in ether at -40°	44.6	18.5	26.1
<i>B</i> Soluble in ether at -40°	100.4	41.8	154.1
<i>C</i> Soluble in acetone at -60°	95.5	39.7	286.4

Fatty-acid composition of lard and cod-liver oil

The component fatty acids of the lard and cod-liver oil which were fed to the animals were determined in a similar manner to that already described for the back fats. Preliminary resolution of the mixed fatty acids was carried out by low-temperature crystallization to give the groups shown in Table 7. The final results of the component acid analyses of the two fats are summarized in Table 8.

DISCUSSION

It is apparent from Table 2 that all four back fats are essentially similar, with the outer-back fat in each case showing, as might be expected, a slightly higher degree of unsaturation. That the fats have undergone oxidation to a considerable extent is shown by their high peroxide values and their low saponification equivalents. The saponification equivalents might have been expected to be intermediate between those of the lard and cod-liver oil which were administered, but in each case the equivalent is even lower than that of the lard, indicating that short-chain acids, derived by oxidative breakdown, are present. However, the saponification equivalent of the hog inner-back fat, when calculated from the determined composition of the two groups of acetone-separated glycerides together with the unsaponifiable matter, gives a value 288.7 which is 10.9 units higher than that found directly on the original back fat (277.8). When the mixed fatty acids were washed with water it was found that repeated extraction failed to remove completely all traces of acidity, indicating a slow removal of acidic products from the mixture.

Successive crystallizations of the hog inner- and outer-back-fat glycerides from acetone at -40° (Tables 3 and 4) resolved each fat into two groups of

glycerides in the proportions of two parts by weight of a fat the iodine value of which approximated closely to that of the lard as fed, to one part of a fatty oil of much greater mean unsaturation. Detailed examination (Table 6) of the fatty acid composition of the acetone-insoluble glycerides of the hog inner-back fat revealed a marked resemblance to that of the lard (Table 8), both in respect of the relative amounts of each acid present and the degree of unsaturation of the C_{14} – C_{18} acids.

Any fat synthesized *de novo* by the animals from carbohydrate and laid down in the depots would be indistinguishable from the lard which was fed (cf. the composition of the inner-back fat of a pig fed on a low-fat diet (Table 8)). Complete separation of lard and biosynthesized back-fat glycerides from cod-liver-oil glycerides by the physical process of crystallization cannot be achieved and the increased content of C_{20} acids (characteristic of cod-liver oil) of a fairly low degree of unsaturation in the acetone-insoluble glycerides is therefore not surprising.

If, in fact, cod-liver-oil glycerides had been absorbed intact, the fatty-acid composition of the acetone-soluble glycerides of the hog inner-back fat would not be expected to reveal a very close resemblance to that of the oil as fed, since it was evident that the back fat was oxidized to a considerable extent; such oxidation would involve loss of unsaturated (polyethenoid) acids. Nevertheless, it is evident from Table 8 that the acetone-soluble glycerides resemble cod-liver oil very strikingly as far as their content of saturated fatty acids is concerned, and that, although the amounts of polyethenoid acids present are much lower than in cod-liver oil, they exhibit unsaturation of a similar high order.

Table 8. Component acids (% w/w) of inner-back solvent-separated glycerides and of lard and cod-liver oil, together with component acids of inner-back fat of pig on low-fat diet

Acid	Hog inner-back fat				
	Inner-back fat (low-fat diet) (Hilditch & Pedelty, 1940)	Lard (present work)	Glycerides insoluble in acetone at -40° (present work)	Glycerides soluble in acetone at -40° (present work)	Cod-liver oil (present work)
Myristic	0.9	0.5	0.5	0.4	1.1
Palmitic	27.5	27.1	32.2	14.9	13.8
Stearic	15.1	15.6	9.0	2.4	2.7
Arachidic	—	0.7	0.2	—	—
Unsaturated C_{14}	0.2	0.4	0.5	1.3	0.4
Unsaturated C_{16}	1.7	2.8	4.0	17.9	8.0
Unsaturated C_{18}	51.5	51.9	48.4	43.9	27.3
Unsaturated C_{20}	3.1	1.0	5.2	14.7	29.4
Unsaturated C_{22}			—	4.5	17.3
Mean unsaturation of					
C_{14} acids	—2.0	—2.0	—2.0	—2.0	—2.0
C_{16} acids	—2.0	—2.1	—2.3	—2.4	—2.3
C_{18} acids	—2.3	—2.3	—2.4	—3.0	—3.3
C_{20} acids	—5.0	—2.3	—4.0	—6.3	—6.3
C_{22} acids			—	—10.3	—7.9

The total percentage of unsaturated fatty acids in the acetone-soluble glycerides (82.3 %) is the same as the corresponding figure (82.2 %) for the cod-liver oil itself, and it is therefore suggested that oxidative splitting of the C_{20} and C_{22} polyethenoid acids occurred giving rise to increased amounts of C_{18} and C_{16} unsaturated acids. We consider that the cod-liver oil was absorbed essentially unchanged from the intestine and laid down in the depots additively along with lard and any fat synthesized by the animal from carbohydrate.

The much discussed question of the mechanism of fat absorption has recently been reviewed by Frazer (1952), who concludes that the intraluminal phase consists of emulsification of triglycerides to yield fine particles (diam. $< 0.05 \mu$) which are then readily absorbed. Emulsification requires a triple combination of fatty acids, partially hydrolysed triglyceride and bile salt, involving a certain degree of lipolysis by pancreatic lipase. While the present work supports the view that glycerides can be absorbed in the unhydrolysed state, it gives no indication of the extent of hydrolysis, though it is evident that it did not take place to any great extent, since the triglycerides which appeared in the depots were identified as a mixture of typical pig fat (lard) and cod-liver oil in an oxidized form.

Aspects of the present study are of interest in relation to the well-known injurious effects of feeding unsaturated fat in a diet low or deficient in vitamin E (see review by Blaxter & Brown, 1952). Manifestations resulting from such a diet vary among species, but include muscular dystrophy, encephalomalacia, oedema and brown coloration of the depot fats; it appears that these conditions are related to the content of unsaturated (mainly polyethenoid) fatty acids in the diet (cf. Dam & Granados, 1945). When the available resources of antioxidant are depleted, oxidation of polyethenoid acids proceeds unchecked, as apparently occurred in the present experiment. It may well be that the lesions produced result from the liberation of plasma-soluble oxidation products (especially from fat depots) which then interfere with normal metabolism in other parts of the body.

That pigs can be raised to bacon weight (200 lb.) on a diet containing 50% of crude whale oil with no overt clinical signs of antioxidant depletion (Walley, 1952) may mean that the polyethenoid acids of this oil are less prone to oxidative attack by virtue of their chemical structure or that the oil itself contains considerably more vitamin E than does cod-liver oil which, according to Brown (1953), contains about 30 mg. α -tocopherol/100 g.

SUMMARY

1. The inner- and outer-back fats of two pigs fed on a diet containing fat (as a mixture of equal parts of lard and cod-liver oil) have been examined. The fats were dark brown and oxidized to a considerable degree.

2. Crystallization of the back fats of one animal from acetone at -40° separated each into 68% of an insoluble glyceride fraction, the iodine value of which approximated to that of lard, and 32% of soluble glycerides of much higher mean unsaturation.

3. Determination of the component fatty acids of the acetone-separated glycerides from the inner-back fat showed that the insoluble glycerides resembled normal pig fat except for the presence of somewhat more unsaturated acids of the C_{16} and C_{20} series than are usually present in the latter. The acetone-soluble glycerides showed a striking resemblance to cod-liver oil in as far as their content of saturated fatty acids was concerned, but contained less polyethenoid C_{20} and C_{22} fatty acids with correspondingly increased amounts of C_{16} and C_{18} unsaturated acids.

4. It was concluded that the lard and cod-liver oil were absorbed essentially unchanged and deposited additively along with typical 'synthesized' pig fat in the depots; it is suggested that depletion of antioxidant (vitamin E) then occasioned oxidation of the polyethenoid fatty acids of the cod-liver oil.

5. The results are discussed in relation to the injurious effects of diets containing unsaturated fat and little or no vitamin E.

We wish to thank Prof. T. P. Hilditch, F.R.S., for his interest in this work and Dr J. A. Lovern (Torry Research Station, D.S.I.R., Aberdeen) for his helpful advice.

REFERENCES

- Blaxter, K. L. & Brown, F. (1952). *Nutrit. Abstr. Rev.* **22**, 1.
Brown, F. (1953). *Nature, Lond.*, **171**, 790.
Dam, H. (1944a). *J. Nutr.* **27**, 193.
Dam, H. (1944b). *J. Nutr.* **28**, 297.
Dam, H. & Granados, H. (1945). *Science*, **102**, 327.
Frazer, A. C. (1952). *Biochem. Soc. Symposia*, no. 9.
Garton, G. A., Hilditch, T. P. & Meara, M. L. (1952). *Biochem. J.* **50**, 517.
Garton, G. A. & Naftalin, J. M. (1953). *Vet. Rec.* **65**, 262.
Gupta, S. S. & Hilditch, T. P. (1951). *Biochem. J.* **48**, 137.
Hilditch, T. P. (1947). *The Chemical Constitution of Natural Fats*, 2nd ed. London: Chapman and Hall.
Hilditch, T. P. & Pedelty, W. H. (1940). *Biochem. J.* **34**, 40.
Walley, J. K. (1952). Ph.D. Thesis, University of Cambridge.