LX. THE INFLUENCE OF LOW AND HIGH PLANES OF NUTRITION ON THE COMPOSITION AND SYNTHESIS OF FAT IN THE PIG

BY THOMAS PERCY HILDITCH, COLIN HENRY LEA
AND WILLIAM HOPPER PEDELTY

*From the Department of Industrial Chemistry, University of Liverpool,
and the Low Temperature Research Station, Cambridge*

(Received 4 January 1939)

THE work described in this communication deals with the determination of the component acids in the depot fats of pigs which had received controlled diets at different levels of nutrition. The primary object was to ascertain how far the quantitative proportions of the various component acids varied in relation to the feeding level of the animal. It was also found possible to make a rough quantitative comparison of the amounts of each fatty acid (*a*) ingested by the animal, (*b*) deposited in the animal in the form of fat, and therefrom to deduce the amount and nature of fat synthesized in the animal, and also to compare the rates of deposition of fat in animals which had been given different rations.

An investigation has recently been in progress at the Animal Nutrition Institute of the School of Agriculture, Cambridge, designed to determine the effect of rate of growth on the proportions of the different parts and tissues in the pig. The physiological and anatomical results of this investigation are in course of publication by Dr C. P. McMeekan. By the courtesy of Dr J. Hammond, specimens of the back and perinephric fats, in sufficient amounts for detailed component acid analyses to be made, were placed at our disposal, together with details of the diets received by the pigs and of the weights of their fatty tissues at the time of slaughter. We are informed by Dr Hammond that the plan of feeding was as follows.

Thirty pigs of unusually homogeneous (inbred) stock were fed on the same diet in different amounts. Up to the age of 16 weeks the pigs were in two groups:

- (*a*) On high plane of nutrition, final weight 120–132 lb. (average 126 lb.).
- (*b*) On low plane of nutrition, final weight 32–37 lb. (average 34 lb.).

Subsequently half of the pigs in each group continued to receive food at the same plane as during the first 16 weeks, whilst the other half were transferred to the alternative level, those on the high plane now receiving the low plane diet, and conversely. The restriction of the ration was that required to make the pigs grow according to pre-arranged growth curves. All these animals were grown to 200 lb. weight before slaughtering.

Details of the rations supplied to the animals were as follows:

	High plane	Low plane
Ration while on sow ...	Free access to sow and to dry meal mixture no. 1; separated milk <i>ad lib.</i>	Restricted access to sow and to dry meal mixture no. 1 and to separated milk
Ration from weaning to 16 weeks ...	1 gal. separated milk per pig daily and meal mixture no. 1 <i>ad lib.</i>	$\frac{1}{2}$ gal. separated milk per pig daily and restricted ration meal mixture no. 1
Ration from 16 weeks onwards ...	1 gal. separated milk per pig daily and meal mixture no. 2 <i>ad lib.</i>	$\frac{1}{2}$ gal. separated milk per pig daily and restricted ration meal mixture no. 2

Composition of meal mixtures

No. 1	%	No. 2	%
Dried separated milk	20	Barley meal	30
White fish meal	30	White fish meal	30
Wheat middlings	30	Flaked meal	30
Flaked meal	20	Wheat middlings	10

The first group of pigs had passed the 16 weeks period before it was decided to reserve sufficient amounts of the back and perinephric fats for the present work, but these specimens were obtained from the final animals killed at 200 lb. weight (pigs nos. 72, 73, 74, 82); a duplicate experiment up to 16 weeks was carried out later from which samples of fat (pigs nos. 138 and 139) were supplied for our analyses.

The weights of fat deposited in the various tissues of each of the animals are summarized in Table I from data kindly supplied to us by Dr C. P. McMeekan (working with Dr Hammond).

Table I. *Total weight, etc., of fat in tissues*

Pig	...	...	...	73 (hog)	74 (gilt)	72 (hog)	82 (gilt)	138 (gilt)	139 (gilt)
Planes of diet	...	...	...	Low-High	High-High	High-Low	Low-Low	High (16 weeks)	Low (16 weeks)
Age (days)	...	...	...	212	181	212	339	112	112
Farm weight (lb.)	...	...	...	204	198	198	197	132	37
Fat in tissues (kg.):									
Subcutaneous				21.69	16.46	15.41	9.80	8.73	0.31
Perinephric and kidney				2.00	1.46	1.50	1.04	0.74	0.02
Intermuscular				6.20	5.96	4.68	4.33	3.27	0.16
Mesenteric				0.95	1.08	0.79	0.93	0.57	0.05
Caul				0.24	0.15	0.11	0.12	0.07	—
Total fat (kg.)				31.08	25.11	22.49	16.22	13.38	0.54
Rate of deposition of fat (g. per diem):									
Subcutaneous				102	91	73	29	78	3
Perinephric				9	8	7	3	7	0.2
Total body fat				147	139	106	48	119	5

Apart from insufficiency of material in certain instances, it was out of the question to carry out detailed fatty acid analyses on each of the 34 depot fats in Table I. Large specimens (1000–1200 g.) of the outer and inner back fats, and of the perinephric fats, were however prepared by extracting the fatty tissues with acetone [cf. Banks & Hilditch, 1932]. After a preliminary analysis of both inner and outer back fats¹ from pig no. 74, which showed differences between the two fats similar to those previously noted [Banks & Hilditch, 1932; Dean & Hilditch, 1933, 2], it was decided to confine detailed examination for the time

¹ I.e. the layers of fat on the inner and outer side of the well-marked dividing line of connective tissue in the subcutaneous fatty layer lying on the thorax and loin of the pig.

being to the outer back fats. This choice was mainly determined by the fact that the unsaturated acids (in which we were particularly interested from the standpoint of keeping qualities of the fat) were known to be present in greater proportions in this section of the fats than in the inner back fat or the perinephric fat. The general characteristics of all the fats which were extracted in quantity are, however, given in Table II.

Table II

Pig no.	Planes of diet	Peri-nephric fat	Inner back fat	Outer back fat		
		I.V.	I.V.	I.V.	Sap. equiv.	Unsap. (%)
73 (hog)	Low-High	52.4	55.6	58.5	288.8	0.2
74 (gilt)	High-High	49.5	54.4*	59.8	285.9	0.2
72 (hog)	High-Low	53.3	57.2	63.3	288.0	0.1
82 (gilt)	Low-Low	57.4	60.4	65.5	286.5	0.1
138 (gilt)	High (16 weeks)	51.0	56.0	60.5	†	†
139 (gilt)	Low (16 weeks)	—	‡	‡	‡	‡

* Sap. equiv. 285.4, unsaponifiable 0.1%.

† No. 138, composite back fat, I.V. 58.0, sap. equiv. 284.9, unsaponifiable 0.1%.

‡ No. 139, composite back fat, I.V. 62.9, sap. equiv. 287.8, unsaponifiable 0.2%.

EXPERIMENTAL

Determination of component fatty acids followed the lines which have been described in recent communications from this laboratory: (a) separation of the mixed fatty acids from about 200 g. of each fat into "solid" and "liquid" acids by the different solubilities of the lead salts in alcohol, and (b) fractional distillation at low pressure of the methyl esters of each group of fatty acids. As a preliminary to the examination of a typical depot fat from each of the six experimental animals, both the inner and outer back fats of the pig which had been fed throughout on the "high" plane of nutrition were studied.

In the case of the inner back fat, separate portions of the esters of the "liquid" acids were distilled by the simple "Willstätter bulb" apparatus, and through an electrically heated and packed column which gives sharper separation of some of the minor components [Longenecker, 1937; Hilditch & Longenecker, 1937]. This showed that the data obtained with the simpler fractionation apparatus, when calculated in terms of all the acids shown to be present by use of the more elaborate column, give results which accord with those from the latter, when dealing with the comparatively simple mixture of fatty acids present in pig and similar depot fats (Table III). Nevertheless the electrically heated column, although it involves a longer period of distillation and also more practice in operation in order to obtain adequate control, gives extremely sharp separation of the successive ester fractions. In the main part of the work the esters of "solid" acids were distilled from a Willstätter bulb, and those of the "liquid" acids through the electrically heated column.

The proportions of the component acids of the inner (Table III) and outer (Table IV) back fats of pig no. 74 have the same relationship to each other as in previously examined specimens of inner and outer back fats from individual pigs [Banks & Hilditch, 1932; Dean & Hilditch, 1933, 2]. The minor component acids are not significantly different, the percentage of palmitic acid is slightly lower in the outer than in the inner back fat, and the chief difference is an increase in the oleic acid content of the outer fat, mainly at the expense of stearic acid.

Table III. *Component acids of inner back fat of pig no. 74*

	“Solid” acids 44.8%	“Liquid” acids 55.2%		Total fatty acids (excluding unsaponifiable matter)			
				% (wt.)		% (mol.)	
		W.	E.H.P.	W.	E.H.P.	W.	E.H.P.
Fractionation apparatus ...	W.						
Myristic acid	0.03	0.47	0.99	0.5	1.0	0.6	1.2
Palmitic acid	27.41	3.62	2.73	31.1	30.1	33.1	32.2
Stearic acid	16.14	—	—	16.2	16.2	15.5	15.5
Tetradecenoic acid	—	—	0.28	—	0.3	—	0.3
Hexadecenoic acid	—	2.88	2.69	2.9	2.7	3.1	2.9
Oleic acid	1.19	39.73	39.65	41.0	40.9	39.7	39.6
Linoleic acid	—	7.10	7.10	7.1	7.1	6.9	6.9
Unsaturated C ₂₀₋₂₂ acids	—	1.27	1.67	1.3	1.7	1.1	1.4
Unsaponifiable	0.03	0.13	0.09	—	—	—	—

W. = esters distilled from a Willstätter bulb.

E.H.P. = “liquid” esters distilled through the electrically heated and packed column.

Constitution of the hexadecenoic acid of pig depot fats. Hilditch & Shorland [1937] found evidence, contrary to previous observations, of the presence of perhaps 3–4 % of hexadecenoic acid in pig depot fats. The present series of analyses show that pigs of about 200 lb. live weight contain 2–3 % of this acid in the mixed acids of the back fats, and the opportunity was taken to ascertain its structure.

A number of the ester fractions, with equivalents ranging from 260 to 280 and i.v. from 40 to 70, obtained from methyl esters of the “liquid” acids of the pig back fats, were united, and their mixed acids submitted to the lead salt-alcohol separation to remove as much palmitic acid as possible. After reconversion of the “liquid” acids thus obtained into methyl esters (28.4 g.), a further distillation through the electrically heated column gave a fraction (11.6 g., sap. equiv. 268.0, i.v. 86.7, CNS value 87.1) which appeared to be substantially methyl hexadecenoate (sap. equiv. 268.0, i.v. 94.8) with minor proportions of methyl palmitate and, perhaps, myristate. (It will be noted that methyl $\Delta^9:10$ -hexadecenoate reacts normally as a monoethenoid derivative with Kaufmann’s thiocyanogen reagent, the thiocyanogen and iodine values being identical.)

Oxidation of this ester in acetone solution with powdered KMnO_4 gave azelaic acid (m.p. and mixed m.p. 101°) and heptanoic acid (Et ester, sap. equiv.: found 154.3, calc. 158.0). The acid in pig depot fat, like that of ox depot fat [Hilditch & Longenecker, 1937], is therefore $\Delta^9:10$ -hexadecenoic acid. This was further confirmed by the production, on oxidation of a dilute alkaline solution of the acid with KMnO_4 , of 9:10-dihydroxypalmitic acid (m.p. and mixed m.p., 125°).

Nature of the highly unsaturated C₂₀₋₂₂ acids present in pig depot fats. Brown & Deck [1930] showed that pig fats contained 0.3–0.4 % of highly unsaturated acids, estimated as arachidonic acid, $\text{C}_{20}\text{H}_{32}\text{O}_2$, from the yield of polybromides from the distilled esters of the lard fatty acids (employing the observed yield of polybromides from methyl arachidonate, 77.6¹). The approximate proportion of C₂₀₋₂₂ acids, estimated from the equivalents of the highest-boiling fractions of the methyl esters, was found to be somewhat larger (ca. 1 %), by Banks & Hilditch [1932] and Dean & Hilditch [1933, 2], whilst in the present series it was uniformly found to be about 2 %.

¹ Subsequently Ault & Brown [1934] gave the yield of insoluble polybromo-adducts from pure methyl arachidonate as 86.5 % of the weight of the ester.

In the present experiments, the polybromo-adducts from accumulated high-boiling fractions from the pig back fats were compared with those from the oil extracted from the fish meal used in the diet of the animals. The polybromo-additive products from each source were debrominated in pyridine solution with zinc [Kaufmann & Mestern, 1936]; the respective products had the following characteristics:

	Acids from debrominated pig fat polybromo- adducts	Acids from debrominated fish meal oil polybromo- adducts
Appearance	Pasty solid	Liquid
Yield from polybromo-adducts	33.8%	27.6%
I.V.	314	336
CNS value	94.5	178

These figures support Brown's observation [1931] that the deposited C_{20-22} acids differ from those ingested as fish oil, and are less unsaturated than the latter; but they do not necessarily imply that arachidonic acid is the only acid concerned. The equivalents of the residual esters left after fractionating the esters of the "liquid" acids (after correcting for any unsaponifiable matter present) were never below that of the ester of a C_{20} acid, and in one case the figure was considerably higher. Since these residual esters must in all cases have still contained some oleate, it follows that some quantity of esters of C_{22} acids was also present.

Component fatty acids of the outer back fats of pigs killed at 200 lb. live weight

About 300 g. of the outer layer of the back fat of each of the four animals fattened to 200 lb. live weight at different planes of nutrition were converted into the mixed fatty acids, which were then resolved into "solid" and "liquid" acids as described earlier. The methyl esters of the "solid" acids were fractionated from a Willstätter bulb, and those of the "liquid" acids through the electrically heated column. The lengthy detailed fractionation data are unnecessary for the subsequent discussion, and only the final results of the analyses need be recorded here. In Table IV the proportions of the component acids are given in the form of both weight and molar percentages.

Table IV. *Component acids of outer back fats (pigs killed at 200 lb. wt.)*

Pig no. ...	73		74		72		82	
Sex ...	Hog		Gilt		Hog		Gilt	
Planes of nutrition* ...	Low-High		High-High		High-Low		Low-Low	
	% wt.	% mol.	% wt.	% mol.	% wt.	% mol.	% wt.	% mol.
Myristic acid	1.1†	1.4†	1.3	1.6	0.7†	0.8†	0.8†	1.1†
Palmitic acid	28.2	30.2	28.3	30.2	25.3	27.2	25.9	27.8
Stearic acid	13.5	13.0	11.9	11.5	13.1	12.7	12.2	11.8
Tetradecenoic acid	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.2
Hexadecenoic acid	2.4	2.6	2.7	2.9	2.0	2.1	2.0	2.1
Oleic acid	47.0	45.4	47.5	46.0	51.0	49.7	48.1	46.8
Linoleic acid	5.2	5.1	6.0	5.8	5.3	5.2	7.8	7.6
Unsaturated C_{20-22} acids	2.4	2.1	2.1	1.8	2.5	2.2	3.0	2.6

* I.e. up to age of 16 weeks, and from 16 weeks to slaughter.

† Includes traces of a lower saturated (lauric?) acid.

*Component fatty acids of composite back fats
of pigs killed at age of 16 weeks*

The determinations were carried out as in the preceding cases, but only about 40 g. were available of the total back fat of the pig fed on the low plane of nutrition. For this reason a specimen of the composite (inner + outer) back fat of each animal was examined in these cases. The final results are given in Table V.

Table V. *Component acids of composite back fats (pigs 16 weeks old)*

Pig no.	...	...	138	139
Sex	...	...	Gilt	Gilt
Plane of nutrition	...	...	High	Low
			% (wt.) % (mol.)	% (wt.) % (mol.)
Myristic acid			1.0*	1.2*
Palmitic acid			29.8	28.1
Stearic acid			12.7	11.8
Tetradecenoic acid			0.2	Trace
Hexadecenoic acid			3.5	4.8
Oleic acid			47.8	42.9
Linoleic acid			3.1	8.2
Unsaturated C ₂₀₋₂₂ acids			1.9	2.9

* Includes traces of a lower saturated (lauric?) acid.

DISCUSSION

It is well recognized that diets rich in fat cause pigs to deposit much of the ingested fat and that, for example, the inclusion of liquid fats rich in oleic and linoleic glycerides, such as cottonseed, maize or groundnut oils, in the ration tends towards the production of soft and oily pig fats [cf. Ellis *et al.* 1926, 1, 2; 1931; 1938]. In the present experiments we are less concerned with the problem of ingested fat than of that synthesized by the animal, since the total fat content of the diets was very low. The fat content of the meal mixtures was 2.2 %, whilst that of the separated milk (which formed the major constituent of all the feeds) was of the order of 0.1 %; the sow's milk taken before weaning contained about 4.5 % of fat. The percentage of fat in the whole of the food taken by the pigs varied somewhat according to the different ratios of separated milk and meals taken in the various experiments, but it never exceeded 1 % of the total diet, the extreme limits being 0.5 % at the "low-low" and 1.0 % at the "high-high" planes of nutrition. Any significant changes in the composition of the depot fats must therefore result almost wholly from differences in the amount of food given to the animal, and not from any sensible alteration in the amount of fat ingested.

We may take in the first place differences in the proportions of the various component acids (Tables IV and V), although these are less notable in percentage form than when the total amount of each component deposited as fat by the animal is considered (cf. below, Table VII). In the group of outer back fats, there is a slight but apparently definite increase in oleic acid content in the case of the 200 lb. weight animals which were fed from 16 weeks onwards on a restricted diet. Parallel with this, the palmitic acid contents in these instances are somewhat lower than in the outer back fats of pigs fattened on the higher plane of nutrition; and this also holds for the feeding at the two dietary levels during the first 16 weeks. In all cases, the variation is within the limits of

$30 \pm 3\%$ (mol.) which have been found to be the characteristic palmitic acid content of the acids of herbivorous animal depot fats [Hilditch & Longenecker, 1937]. The stearic acid contents of all six outer back fats showed little variation.

Of the minor components, it will be seen that only traces of saturated acids of lower molecular weight than myristic acid are present. Hexadecenoic (and traces of tetradecenoic) acids are fairly constant at 2.0–2.7% in the fats of the 200 lb. weight animals, but somewhat higher at the 16 weeks' stage, irrespective of the plane of nutrition. The unsaturated C_{20-22} acids are more prominent than has been observed in previous cases; and in this connexion it should be noted that the proportion of fish meal, 10–13% of the total diets, was fairly considerable. Woodman *et al.* [1937] state that inclusion of 5% of fish meal in the ration up to the time of slaughtering involves a risk of producing a fishy taint in bacon.

The most variable of the minor constituents is linoleic acid. In fats from 200 lb. weight animals fattened on the high plane diet, this formed about 5–6% of the fatty acids, whilst at 16 weeks (high plane) it was only 3%; but it reached 8% of the total outer back fatty acids of the animals fed on the restricted ration, either at 16 weeks or throughout until the pig had reached 200 lb. live weight. This variation is significant in regard to the relative softness of the depot fats (and, possibly, their keeping quality; although in the latter respect other factors may also come into play). Differences in linoleic acid content, though small numerically, can have an important effect on the glyceride structure of the fat [Green & Hilditch, 1938]: linoleic acid, as a minor component, will not be present more than once in any triglyceride molecule, whereas oleic acid, the most prominent component, will be present in the majority of the triglyceride molecules. Hence any linoleic acid is almost certain to be present as a linoleo-oleo-glyceride, and of course glycerides containing two unsaturated acyl groups are liquid at the ordinary temperature. Consequently, whilst a content of 5% of linoleic acid connotes about 15% of corresponding liquid glycerides, 8% of linoleic acid involves the presence of about 24% of liquid glycerides—nearly 10% more of the fat being of a type of lower melting point in consequence of an increase of 3% in the linoleic acid percentage. The increases observed, at low planes of nutrition, in the linoleic, and also the oleic, acid contents of the depot fats are clearly the cause of the already known fact [Ellis *et al.* 1925; 1930; Robison, 1931; Callow, 1937] that rapid growth of pigs (i.e. increased rate of fat deposition) leads to the production of firmer fat.

Apart from increased tendency to softness of fat, the uneconomic rate of production of fat at low as compared with higher planes of nutrition is clearly evident from the data in Table I.

The variations in the rate of fat deposition, and also in the mean unsaturation of the fats (so far as can be judged (Table II) from i.v. alone), are parallel in all the depots of the animal. The most rapid rates of production, and more saturated (i.e. firmer) fats, result when the diet after the first 16 weeks is on a high plane of nutrition. Abundant diet is more important during these latter stages of growth than during the first 16 weeks of life. As a matter of fact, the most rapid production, but not quite the firmest type, of fat resulted from restricted diet up to 16 weeks, followed by the high plane of nutrition for the remainder of the life of the animal. A "low plane" diet up to 16 weeks followed by "high plane", again, gives much better results both as regards rate of fat production and relative saturation of the fat than the alternative of "high plane" diet up to 16 weeks and then a restricted diet to 200 lb. weight.

It should be noted that these conclusions refer only to fat formation in the animal, and take no account of bone and muscle production. Thus, although the fat is softer and produced more slowly in the animal fed on the "high-low" planes of nutrition than in that fed on the "low-high" planes, the former animal is of more desirable conformation as a whole—long and lean—than the latter which is of the short, fat type.

The effects of restricted rations throughout the whole life of the pig are seen to be very uneconomical in the long period taken to reach 200 lb. live weight, the excessive consumption of food required to produce a given increase in body weight, and in the relatively unsaturated and soft character of the resulting depot fats.

The component acid data discussed above may be utilized to yield some information of an approximate order as to the amount of each individual fatty acid deposited by the pig, and to compare these figures with the corresponding amounts of the fatty acids consumed by each animal in its diet. Obviously, a number of approximations and estimates have to be made in order to obtain all the necessary data, but we have satisfied ourselves that any errors thereby introduced are insufficient to alter the general conclusions to be drawn later from this comparison. The computation of individual acids in the fats of the diet is that most liable to errors in estimation, but the total quantity of fat ingested by any animal is so much less than that deposited in the animal, that wider variations from our estimates of the component acids present than are at all likely would have only minor effects on the ultimate comparison between ingested and deposited fats. The following methods have been used in these calculations.

Component acids ingested as fat in the diet. The amount of each individual fatty acid combined as fat in 100 parts of each of the constituents of the food given to the pigs has been calculated with the results shown in Table VI. The component acid data used for the dietary fats have been taken from the following sources:

Sow's milk fat. The figures are from a partial analysis (not using ester-fractionation) [Laxa, 1931].

Cow's milk fat. The data obtained by ester-fractionation for a typical cow's milk fat of similar i.v. by Dean & Hilditch [1933, 1] have been used, but modified to include the 5 % of lower unsaturated acids now known to be present [Hilditch & Thompson, 1936; Longenecker & Hilditch, 1938].

White fish meal oil. The component acids are taken from analyses by Lovern [1937] for halibut and turbot flesh fats (no others except herring being available). If the C_{20-22} acid contents in other fish flesh fats should be somewhat lower, such difference will not affect the conclusions drawn from Table VII (below).

Wheatmeal, maize and barley oils. The fatty acid data are based on recent determinations by modern methods respectively by Sullivan & Bailey [1936], Baughman & Jamieson [1921], and Täufel & Rusch [1929]. The i.v. of the meal oil differed somewhat from those of the oils analysed by these workers, i.e. the proportions of oleic and linoleic acids are not exactly those given. It is hardly legitimate, in the case of these seed fats, to adjust the proportions of these acids according to the i.v. of the fats (*vide infra*), but here again alternative calculations have shown that the differences thereby introduced have no significance in regard to the final comparison. In accordance with recent observations [Hilditch & Jasperson, 1938], however, 1 % of hexadecenoic acid has been allowed in each case.

Component acids deposited as fat in the pigs. The data in each case depend upon our determinations of the acids in the outer back fats. Since it is now well

Table VI. *Approximate amounts of fatty acids present as fat in the dietary constituents*

Fatty acids	Sow's milk %	Separated milk %	Meal mixtures	
			No. 1 %	No. 2 %
Saturated:				
Below C ₁₄	0.04	0.011	0.02	—
Myristic	0.13	0.010	0.05	0.03
Palmitic	1.21	0.024	0.27	0.22
Stearic	—	0.007	0.06	0.05
Unsaturated:				
C ₁₆ (and C ₁₄)	—	0.005	0.06	0.05
Oleic	2.94	0.034	0.58	0.63
Linoleic (and linolenic)	—	0.004	0.79	0.89
C ₂₀₋₂₂	—	—	0.24	0.24
	4.32	0.095	2.07	2.11

established that, within the fats of a single animal, or indeed over wider ranges of pig, ox and sheep depot fats, difference in total unsaturation is very largely conditioned by respective differences in the contents of oleic and stearic acids [cf. Tables III and IV; Banks & Hilditch, 1931; 1932; Dean & Hilditch, 1933, 2], it is quite safe in these cases, for small differences in i.v., to adjust the stearic and oleic acid contents determined on one of the depot fats of the animal concerned. This has been done in the case of each pig in the present series. The i.v. of the inner and outer back fats and the perinephric fats are given in Table II; the intermuscular fats were assumed to have similar i.v. to the inner back fats, and the mesenteric and caul fats to have similar i.v. to the perinephric fats. From the total weights of fat in each tissue (Table I) the individual acids deposited as fat were then calculated.

Table VII shows the weights (kg.) of each individual acid or group of acids thus calculated to have been (a) received as fat in the food or (b) deposited as fat

Fig. 1. Ingestion and deposition of fatty acids by pigs. Fatty acids: ingested, white; deposited in tissues, black. L, lauric (and lower saturated) acids; M, myristic acid; P, palmitic acid; S, stearic acid; H, hexadecenoic acid; O, oleic acid; Ln, linoleic acid; A, unsaturated C₂₀₋₂₂ acids.

in the tissues of the four experimental animals fattened to 200 lb. live weight and of the two animals killed at the age of 16 weeks. Fig. 1 is a graphical presentation of the same data.

Table VII. *Comparison of acids in fat ingested and deposited by pigs*

(a) Pigs at different planes of nutrition to 200 lb. wt.

Pig	73 (Hog)			74 (Gilt)		
Planes of nutrition ...	Low-High			High-High		
Fatty acids (kg.)	Fed	Deposited	Difference	Fed	Deposited	Difference
Saturated:						
Below C ₁₄	0.11	Trace	-0.11	0.11	—	-0.11
Myristic	0.24	0.29	+0.05	0.26	0.24	-0.02
Palmitic	1.28	8.27	+6.99	1.53	6.92	+5.39
Stearic	0.25	3.99	+3.74	0.24	3.58	+3.34
Unsaturated:						
C ₁₆ (and C ₁₄)	0.22	0.89	+0.67	0.22	0.72	+0.50
Oleic	3.24	13.87	+10.63	3.80	10.50	+6.70
Linoleic	3.30	1.48	-1.82	3.22	1.43	-1.79
C ₂₀₋₂₂	0.87	0.74	-0.13	0.86	0.48	-0.38
	9.51	29.53	+20.02	10.24	23.87	+13.63

Pig	72 (Hog)			82 (Gilt)		
Planes of nutrition ...	High-Low			Low-Low		
Fatty acids (kg.)	Fed	Deposited	Difference	Fed	Deposited	Difference
Saturated:						
Below C ₁₄	0.12	Trace	-0.12	0.16	Trace	-0.16
Myristic	0.24	0.21	-0.03	0.27	0.15	-0.12
Palmitic	1.36	5.45	+4.09	1.28	4.00	+2.72
Stearic	0.20	2.99	+2.79	0.25	2.16	+1.91
Unsaturated:						
C ₁₆ (and C ₁₄)	0.17	0.43	+0.26	0.22	0.31	+0.09
Oleic	3.27	10.69	+7.42	3.07	7.09	+4.02
Linoleic	2.44	1.07	-1.37	2.85	1.23	-1.62
C ₂₀₋₂₂	0.66	0.53	-0.13	0.74	0.46	-0.28
	8.46	21.37	+12.91	8.84	15.40	+6.56

(b) Pigs at different planes of nutrition to 16 weeks

Pig	138 (Gilt)			139 (Gilt)		
Planes of nutrition ...	High			Low		
Fatty acids (kg.)	Fed	Deposited	Difference	Fed	Deposited	Difference
Saturated:						
Below C ₁₄	0.08	Trace	-0.08	0.02	—	-0.02
Myristic	0.14	0.13	-0.01	0.04	0.01	-0.03
Palmitic	0.87	3.81	+2.94	0.32	0.15	-0.17
Stearic	0.07	1.14	+1.07	0.01	0.06	+0.05
Unsaturated:						
C ₁₆ (and C ₁₄)	0.07	0.51	+0.44	0.01	0.03	+0.02
Oleic	1.97	6.48	+4.51	0.75	0.23	-0.52
Linoleic	0.73	0.38	-0.35	0.08	0.04	-0.04
C ₂₀₋₂₂	0.21	0.25	+0.04	0.02	0.01	-0.01
	4.14	12.70	+8.56	1.25	0.53	-0.72

It is an interesting point that the only animal which did not deposit more fat than it ingested is the 16-weeks old pig fed on a restricted ration. At the same time, it will be seen that, even at this very low plane of nutrition, obvious fat synthesis took place during the subsequent stages of growth. The fat deposition in the four 200 lb. weight pigs and in the 16-weeks old pig fed on the high plane of nutrition is not only well marked, but follows the same lines in each case as regards the individual fatty acids which have been laid down.

The amounts of palmitic, oleic and stearic acids in the deposited fats greatly exceed those ingested in the respective diets, and show that the fat synthesized by the animal from non-fatty sources consists predominantly of glycerides of these three acids. The same conclusion applies to the hexadecenoic acid in the depot fats which, though relatively small in amount, is usually several times as much as can possibly have been present in the dietary fat. Myristic acid, however, is present in about the same amount in the animal fats as in the ingested fats, and the evidence as to its origin is therefore inconclusive; unless the absorption of myristic glycerides from the diet was extremely complete, some of this acid must also have been synthesized.

The remaining pig fat component acids—linoleic acid, the unsaturated C_{20-22} acids and saturated acids below myristic—show an opposite relationship to the respective ingested fatty acids: the amount deposited is consistently less than that present in the diet. It will be noticed that, both in this group and in the preceding group (where evidence of fat synthesis is pronounced), the respective differences for each of the animals show, on the whole, a remarkable and often almost quantitative degree of similarity.

It is clear that fats containing saturated acids of lower molecular weight than myristic acid are neither synthesized nor assimilated by the pig; this accords with the observations of other workers on the body fats of rats and some other animals.

The quantity of linoleic acid in the body fats is not more than (and usually definitely less than) half of that available in the dietary fats; this suggests strongly, although it does not afford positive proof, that, like the rat, the pig is not able to synthesize linoleic acid and derives glycerides of this acid only by assimilation. This view may receive further support from the fact that the octadecadienoic acid of pig depot fats, unlike that of ox depot fat [Hilditch & Longenecker, 1937] has the properties of seed-fat linoleic acid [Hilditch & Stainsby, 1935]. Ellis & Zeller [1930] similarly concluded that, in the pig, linoleic acid is not synthesized, but comes from the food, except possibly in the case of young animals.

The quantity of unsaturated C_{20-22} acids present as glycerides in the depots likewise falls short of that present in the diet (in this instance in the fish meal constituents); but the disparity is less pronounced than in the case of linoleic acid, and the possibility of some slight degree of synthesis of the acids of this group by the pig (especially in the first weeks of its life) cannot be excluded. For the most part, however, they seem to be derived from the fish meal present in the diet. It was pointed out earlier that the highly unsaturated C_{20-22} acids in the pig differ in properties from the corresponding acids of the fish meal, and selective absorption of some of the group is perhaps an alternative possibility.

Whilst it cannot, of course, be taken for granted that the increases recorded in Table VII in palmitic, stearic and oleic acids represent the total amounts of each of these acids which have been synthesized, it is of interest to note that the ratios of the increases of palmitic to those of the two C_{18} acids are as follows:

Pig no.	Palmitic	Stearic + oleic	Palmitic : C_{18} acids
73	6.99	14.37	1 : 2.06
74	5.39	10.04	1 : 1.86
72	4.09	10.21	1 : 2.50
82	2.72	5.93	1 : 2.18
138	2.94	5.58	1 : 1.90

The mean ratio for the whole series is 1:2.08 (wt.) or 1:1.89 (mol.). Palmito-di- C_{18} glycerides (especially palmitodiolein and palmito-oleostearin) have

previously been shown to be the main components of pig depot fats [Hilditch & Stainsby, 1935], and the present figures point to these as the predominant glycerides in the fat produced by synthesis in this animal.

SUMMARY

The component acids of a series of depot (back) fats from pigs fed on different planes of nutrition have been determined. On a restricted diet the deposition of fat is not only slower, but the fat produced is softer, owing to increase in the small proportions of linoleic acid present, together with some increase in the proportion of oleic acid.

Comparison of the amounts of each component acid in the total fats present in the diets with those in the body fats of the animals shows substantial synthesis of glycerides of palmitic, oleic and stearic acids, in the average proportion of 1 mol. palmitic to 1.9 mol. C_{18} acids. The minor components hexadecenoic and (possibly) myristic acid may also be mainly formed by synthesis, but linoleic and unsaturated C_{20-22} acids in the depots are derived only from ingested fats.

Our cordial thanks are offered to Dr J. Hammond for his courtesy in providing the experimental material and allowing us to consult his data as regards the diet and the composition of the tissues of the animals, and to Dr E. H. Callow for assistance in the extraction and the determination of the general characteristics of the fats in the feeding-stuffs and in some of the pig depots. The work described in this communication forms part of a research programme undertaken by the Food Investigation Board of the Department of Scientific and Industrial Research, to whom we are indebted for permission to publish the results.

REFERENCES

- Ault & Brown (1934). *J. biol. Chem.* **107**, 615.
 Banks & Hilditch (1931). *Biochem. J.* **25**, 1168.
 ——— (1932). *Biochem. J.* **26**, 298.
 Baughman & Jamieson (1921). *J. Amer. chem. Soc.* **43**, 2696.
 Brown (1931). *J. biol. Chem.* **90**, 133.
 ——— & Deck (1930). *J. Amer. chem. Soc.* **52**, 1135.
 Callow (1937). *Rep. Food Invest. Bd, Lond.*, p. 43.
 Dean & Hilditch (1933, 1). *Biochem. J.* **27**, 889.
 ——— (1933, 2). *Biochem. J.* **27**, 1950.
 Ellis (1938). *Oil & Soap*, **15**, 66.
 ——— & Hankins (1925). *J. biol. Chem.* **66**, 101.
 ——— & Isbell (1926, 1). *J. biol. Chem.* **69**, 219.
 ——— (1926, 2). *J. biol. Chem.* **69**, 239.
 ——— Rothwell & Pool (1931). *J. biol. Chem.* **92**, 385.
 ——— & Zeller (1930). *J. biol. Chem.* **89**, 185.
 Green & Hilditch (1938). *J. Soc. chem. Ind., Lond.*, **57**, 49.
 Hilditch & Jaspersen (1938). *J. Soc. chem. Ind., Lond.*, **57**, 84.
 ——— & Longenecker (1937). *Biochem. J.* **31**, 1805.
 ——— & Shorland (1937). *Biochem. J.* **31**, 1499.
 ——— & Stainsby (1935). *Biochem. J.* **29**, 90.
 ——— & Thompson (1936). *Biochem. J.* **30**, 677.
 Kaufmann & Mestern (1936). *Ber. dtsch. chem. Ges.* **69 B**, 2684.
 Laxa (1931). *Ann. Falsif.* **24**, 87.
 Longenecker (1937). *J. Soc. chem. Ind., Lond.*, **56**, 199 T.
 ——— & Hilditch (1938). *J. biol. Chem.* **122**, 497.
 Lovern (1937). *Biochem. J.* **31**, 755.
 Robison (1931). *Bull. Ohio agric. Exp. Sta.* no. 152, 184.
 Sullivan & Bailey (1936). *J. Amer. chem. Soc.* **58**, 383, 390.
 Täufel & Rusch (1929). *Z. Untersuch. Lebensmitt.* **57**, 422.
 Woodman, Evans & Turpitt (1937). *J. agric. Sci.* **27**, 569.