7. THE INFLUENCE OF PROLONGED STARVATION ON THE COMPOSITION OF PIG DEPOT FATS

By THOMAS PERCY HILDITCH and WILLIAM HOPPER PEDELTY

From the Department of Industrial Chemistry, University of Liverpool

(*Received 9 November 1939*)

Until recently, little or no information has been published with reference to the rates at which the individual component acids of a depot fat disappear during starvation. Work in this field of a strictly quantitative nature is lacking except for recent studies by Longenecker [1939, 1, 2, 3] detailed below. Dowell [1920] raised rabbits on a peanut diet for 40 days, after which some were killed and others were starved for periods from 3 to 7 days. His results showed a fall in I.V. for the depot fats and also a preferential selection of the back fat over that surrounding the kidney. A similar diminution in I.V. was noted by Stefko [1928] in the subcutaneous and deeper fat tissue of tubercular and starved human subjects. Mann [1936] also showed that fat changes in hibernating bats were characterized by a rise in M.P. with corresponding fall in mean unsaturation. Lovern [1934; 1938] found that in the fasting herring the more unsaturated components of the fat appeared to be preferentially utilized; but in fasting salmon the average unsaturation was not greatly altered, except for some decrease in hexadecenoic acid. Frank [1926] concluded that in starved rats desaturation of saturated fatty acids accompanies β-oxidation of fatty acids.

The only other studies of the effect of inanition on depot fat composition are those of Longenecker on the rat. Longenecker [1939, 1] found that the composition of the total body fat of a normal rat after 28 % loss in weight during starvation showed little significant alteration; a similar strain of animal after rearing on a high carbohydrate diet with subsequent starvation to 22 % weight loss gave a body fat, which, when compared with the control, showed a decrease in total C_{16} acids (palmitic and hexadecenoic) with a corresponding rise in the amount of oleic and linoleic acids. In a second group of experiments [1939, 2], rats were fed on a high corn oil diet which produced a "soft" fat. Two groups were then fasted to 14 and 24 % weight loss and the results showed that utilization (during inanition) of rat depot fat containing mainly oleic and linoleic acids did not produce marked changes in the relative proportions of these acids, little selection being evident from the available fatty acid mixture. A gradual increase in oleic acid content parallel with reduction in linoleic acid content occurred during fasting, but this might be regarded as within the limit of physiological variation rather than as an indication of a preferential utilization of the more highly unsaturated acids. In a third paper, Longenecker [1939, 3], describes similar fasting of rats fed for 14 days on a diet rich in coconut oil. After fasting to 15 % loss in weight, lauric acid disappeared more rapidly than oleic acid; on further fasting to 30 % loss in weight the same effect was observed, although the ratio of oleic : lauric acid utilized was higher at this stage. Longenecker points out that, whilst his data may indicate preferential utilization of acids of

lower mol. wt., they may equally be attributed to the random removal of triglyceride molecules from the depots, since most of these molecules contain two lauric (or other lower saturated acyl) groups, the third being oleic, myristic or palmitic.

The present investigation was undertaken to ascertain whether, during extreme starvation of pigs, any preferential utilization of individual saturated or unsaturated fatty acids occurs in the depot fats. Of all domestic animals the pig is the most suitable for studies of this nature, on account of its relatively large proportion of reserve fat. Attention was confined to the outer and inner back fats and the perinephric fats, which were clearly likely to undergo most marked alteration, since during starvation an animal uses its depot fats in preference to the more vital organ fats, the latter only being appreciably utilized in the final stages preceding death.

Experimental

As in our recent study of the deposition of component glycerides in back and perinephric fats of the pig [Hilditch *et al.* 1939], the present examination was made possible by access to the depot fats of pigs from an experiment conducted by Dr J. Hammond at the Animal Nutrition Institute of the School of Agriculture, Cambridge, on the changes occurring during fasting. This experiment consisted in rearing 5 pigs of homogeneous stock and sex (hogs) from weaning as rapidly as possible to approximately 330 lb. live weight on an unrestricted diet consisting of the following meal mixture: white fish meal 15%, flaked maize 40%, barley 20% and pollards 25%, with the addition of 1% of cod liver oil. One animal (no. 175) served as control and was killed as soon as it reached 330 lb. live weight; the other four were fasted (except for the provision of water containing essential mineral salts) and given exercise daily. Of these animals, only the fats of the 3rd and 5th animals to be killed have been studied in detail in the course of the present work. The pigs in question were no. 167, starved for 51 days, and the final animal, no. 173, which had fasted for 135 days and was quite active and apparently healthy at the time of death. Both animals lost weight much more slowly than had been expected and it is considered that pig

Table 1

Pig	Days fasted	Killing weight lb.	Loss in weight lb.	% loss in weight	Total weight of left loin and thorax g.	Weight of both perinephric fats g.
175	0	327	0	0	6671·5	3806·0
167	51	263	65	19·8	4149·0	2878·0
173	135	188	143	43·2	1754·3	1171·0

		Fresh tissue				Extracted fat		
Pig	Depot	Weight g.	Fat %	Tissue %	Water %	Weight g.	I.V.	% of pig 175
175	Outer	2928·0	87·1	2·2	10·7	2549	63·9	100
	Inner	3743·5	92·7	1·2	6·1	3472	58·9	100
	Perinephric	3806·0	93·5	1·2	5·3	3557	56·4	100
167	Outer	1729·0	87·1	2·9	10·0	1513	65·0	59·4
	Inner	2420·0	91·2	1·5	7·3	2208	59·9	63·6
	Perinephric	2878·0	93·0	1·3	5·7	2676·5	56·6	75·3
173	Outer	854·3	82·7	4·3	13·0	706·5	60·0	27·7
	Inner	900·0	86·3	2·6	11·1	776·7	54·7	22·4
	Perinephric	1171·0	87·4	2·8	9·8	1023·4	54·8	28·8

no. 173 would have lived considerably longer had time permitted. By the kind co-operation of Dr Hammond, we were supplied with the foregoing data and with the weights of fatty tissues, etc., at the time of slaughter as set out in Table 1. The tissues, from which the depot fats examined in this work were prepared, were handed to Drs E. H. Callow and C. H. Lea of the Low Temperature Research Station, Cambridge, who very kindly undertook the extraction (and storage at $-20°$ until required) of the fats dealt with in the analyses now recorded.

The component acids of the 9 depot fats from the 3 pigs (nos. 175, 167 and 173) were determined as already described [Hilditch *et al.* 1939]. After the preliminary division into "solid" and "liquid" acids by Pb salt separation from alcohol, the methyl esters of the "solid" acids were distilled from a Willstätter flask, and those of the "liquid" acids through an electrically-heated and packed column of the type described by Longenecker [1937]. The final weight and molar percentages determined for the acids from each depot fat are given in Table 2.

Table 2. *Component acids of control and starved pigs*

(*a*) Weight % (excluding unsaponifiable matter)

	Pig 175			Pig 167			Pig 173		
Acid	Outer	Inner	Peri-nephric	Outer	Inner	Peri-nephric	Outer	Inner	Peri-nephric
Lauric	0·1	0·1	—	—	—	0·1	Trace	0·1	Trace
Myristic	0·9	0·8	0·9	0·9	0·6	0·8	0·9	0·9	0·9
Palmitic	26·5	27·5	29·3	26·0	29·4	31·3	30·1	30·7	30·3
Stearic	12·8	15·1	17·4	13·0	15·0	17·6	15·1	18·8	21·5
Tetradecenoic	0·2	0·2	0·3	0·2	0·2	0·1	0·2	0·2	0·2
Hexadecenoic	1·9	1·7	1·8	1·7	2·4	1·0	2·6	1·7	2·2
Oleic	46·8	44·2	40·3	45·6	40·0	38·8	39·5	37·2	34·1
Linoleic	7·9	7·3	8·1	9·1	9·6	8·3	8·2	7·1	7·3
Unsaturated C_{20-22}	2·9	3·1	1·9	3·5	2·8	2·0	3·4	3·3	3·5
(*b*) Molar % (excluding unsaponifiable matter)									
Lauric	0·1	0·2	—	—	—	0·2	Trace	0·2	Trace
Myristic	1·0	0·9	1·0	1·1	0·7	0·9	1·0	1·1	1·0
Palmitic	28·4	29·5	31·3	27·9	31·4	33·3	32·2	32·7	32·4
Stearic	12·3	14·6	16·7	12·6	14·5	16·9	14·5	18·1	20·7
Tetradecenoic	0·2	0·2	0·4	0·2	0·2	0·1	0·3	0·3	0·2
Hexadecenoic	2·1	1·8	1·9	1·9	2·6	1·1	2·8	1·8	2·4
Oleic	45·6	42·9	39·1	44·4	38·8	37·6	38·2	36·0	33·1
Linoleic	7·7	7·2	7·9	8·9	9·4	8·1	8·0	6·9	7·1
Unsaturated C_{20-22}	2·6	2·7	1·7	3·0	2·4	1·8	3·0	2·9	3·1

Discussion

If we consider, in the first place, some of the more general data in Table 1, it will be seen that as fat is withdrawn from the depots to meet energy requirements the relative amount of tissue and water in the fatty tissue increases accordingly. The earlier stages of inanition show a preferential selection from the reserves of the outer back fat with a marked sparing of the more vital perinephric fat. Prolonged starvation, however, causes the largest degree of mobilization to be found in the inner back fat whereas the perinephric fat has been reduced in almost the same proportion as the outer back depots.

Preliminary investigation of possible changes in the nature of the depot fat, as far as can be judged from I.V. alone, seems to indicate that during the course of starvation the animal mobilizes its fat in a different way at later stages.

From Table 1 it can be seen that after 51 days' fasting each of the depots shows an increase in I.V. over those from the control, while after 135 days the reverse has happened—in all cases the depots are considerably more saturated in character than those in the control animal. There occurs then, at some stage of the fasting period a point of maximum I.V. (of the whole fats), after which the animal commences either to select preferentially, or (less probably) to saturate, unsaturated acids.

On the other hand, a preliminary examination of Table 2 shows that on the whole remarkably little difference in the proportions of component acids has accompanied prolonged starvation. In the first period of inanition the stearic acid content of all three depots shows little change whereas the other major saturated component acid (palmitic) has increased in the cases of the inner back and perinephric fat but shows a slight decrease in the outer back fat. Again, oleic acid has decreased in amount in each depot but there have been appreciable increases in linoleic and unsaturated C_{20-22} acids,[1] especially in the outer and inner back fat. The largest differences, taken all round, are to be found in the depots of the back, and this is in accordance with the previous observation, from Table 1, of the preferential mobilization of these reserves during the first period of starvation. It seems obvious that there has been some degree of selection of unsaturated fatty acids from the increased amount of linoleic acid in all depots. Limits in experimental technique forbid speculation as to changes occurring in the other minor component saturated and unsaturated acids except to say that in the second stage of starvation selection of fatty acids has produced a slight rise in the proportions of hexadecenoic acid.

The second stage of starvation has produced the following results common to all three depots: considerable mobilization of both unsaturated acids of the C_{18} series with parallel increases in the content of stearic acid; palmitic acid has found a common level at 32–33 % (mol.); definite increases in the content of unsaturated C_{20-22} acids. We also find that after this drastic starvation the perinephric fat, judging from Table 2 alone, has been mobilized to the same extent as other reserves (an observation also confirmed by Table 1).

One must not lose sight of the fact that the units concerned in withdrawal of fat from the tissues are not individual fatty acids but triglyceride molecules; this will tend to diminish the spectacular nature of results in the event of preferential selection. Taking, as a case in point, the highly unsaturated acids of the C_{20} and C_{22} series present as minor components in pig depot fats, it is now recognized that no triglyceride molecule will contain more than one of these particular acyl groups (whilst in many others they will not be present at all). Let us suppose, by way of example, that these highly unsaturated C_{20-22} acids are preferentially selected; then, unless immediate resynthesis into glyceride takes place, the accompanying fatty acids will also be mobilized. Again, it should be remembered that the accompanying fatty acids will probably be a heterogeneous mixture of all the other acids present in the fat. Preselection of other acids may also occur and the picture of the process which is happening becomes extremely complex. Great caution is therefore necessary in interpreting the results given in Tables 1 and 2.

The experimental material at our disposal has furnished detailed statements of the component acids of the depot fats of pigs which have been fasted for very prolonged periods under conditions in which their metabolic processes were as little disturbed as possible. The lengthy periods of inanition should have per-

[1] The high proportion of this group of acids in the control animal is undoubtedly due to the high proportion, 15 %, of fish meal and the daily dose of cod liver oil in the diet.

mitted the changes in the depot fats to develop more fully than otherwise; moreover, the loss of fat from the depots is considerably greater than in most other previous studies of this kind—the pig fasted for 135 days had lost nearly half its original live weight and over 70 % of its original back and perinephric fats. Nevertheless the most striking feature of the results is the relatively small change in composition of the fats in corresponding depots during the course of prolonged starvation; selective mobilization of certain fatty acids is to be observed, but this effect is subsidiary and of quite small magnitude.

By combining the data in Tables 1 and 2 it is possible to construct an approximate balance sheet of the total amount of each fatty acid actually present in each depot of the animals before and after starvation. The final results so obtained for the weights of each component acid present in each of the pig depot fats are shown in Table 3.

Table 3. *Total amounts of fatty acids in pig depots (g.)*

	Pig 175 (Control)			Pig 167 (51 days fasted)			Pig 173 (135 days fasted)		
Acid	Outer	Inner	Peri-nephric	Outer	Inner	Peri-nephric	Outer	Inner	Peri-nephric
Lauric	2	3	—	—	—	3	—	1	—
Myristic	22	26	30	13	13	20	6	7	9
Palmitic	641	907	990	374	616	796	202	227	295
Stearic	310	498	588	187	315	447	101	139	209
Tetradecenoic	5	7	10	3	4	3	1	1	2
Hexadecenoic	46	56	61	24	50	25	17	13	21
Oleic	1133	1458	1362	655	839	986	265	274	332
Linoleic	191	241	274	131	201	211	55	52	71
Unsaturated C_{20-22}	70	102	64	50	59	51	23	24	34
Total	2420	3298	3379	1437	2097	2542	670	738	973
Total saturated	975	1434	1608	574	944	1266	309	374	513
Total unsaturated	1445	1864	1771	863	1153	1276	361	364	460
Total C_{16}	687	963	1051	398	666	821	219	240	316
Total C_{18}	1634	2197	2224	973	1355	1644	421	465	612

Table 3 should be studied, not with respect to the actual amounts of acids present in the tissues, but in relation to the changes in amount of each acid produced during starvation. These changes become clearer if the % of each acid remaining at each of the two stages of fasting are calculated from Table 3, with the results given in Table 4.

Table 4 is confined to data for the major and more important minor component acids. In the case of the second period, i.e. 84 days, the figures represent the proportions of acids at the 51 days stage and not those present in the original control animal. Certain new facts come to light on examining this Table. In the first 51 days of starvation the amount of stearic acid remaining followed closely the proportion of total fatty acids which was unmobilized, whereas in the case of linoleic acid the proportion mobilized was considerably less for all three depots than the corresponding amounts for the total fatty acids. This can only be interpreted to mean that in the early stages of inanition there is considerable sparing of linoleic acid. Comparing figures for this acid and the corresponding total fatty acids in the depots for the subsequent 84 days of starvation we find that linoleic acid has not been spared but slightly preferentially metabolized. Right throughout the whole period of 135 days more unsaturated than saturated acids have disappeared and also acids of the C_{18} group

Table 4. *Percentage proportions of fatty acids remaining in pig depots after inanition*

	First 51 days			Subsequent 84 days		
Acid	Outer %	Inner %	Peri-nephric %	Outer %	Inner %	Peri-nephric %
Palmitic	58	68	80	54	37	37
Stearic	60	63	76	54	44	47
Hexadecenoic	52	89	41	71	26	84
Oleic	58	58	72	40	33	34
Linoleic	69	83	77	42	26	34
Unsaturated C_{20-22}	71	58	80	46	41	67
Total saturated	59	66	79	54	40	41
Total unsaturated	60	62	72	42	32	36
Total C_{16}	58	69	78	55	36	38
Total C_{18}	60	62	74	43	34	37
Total fatty acids	59	64	75	47	35	38

Note. This table should be interpreted in the following manner: The figures in each column represent the % of fatty acid unmobilized and should be compared with that at the foot of the Table. When the figure for an individual or group of fatty acids is higher than that at the foot, it is an indication that sparing has taken place; a lower figure therefore indicates preferential mobilization.

have been selected in preference to C_{16} acids; in connexion with this latter observation it will be seen that oleic acid, the most prominent component of pig fat, has suffered larger reductions than any other acid. There is marked sparing of the unsaturated C_{20} and C_{22} acids (although the data are slightly erratic); hexadecenoic acid shows similar sparing in some, but not all, cases. The results for these two groups of acids should however be treated with caution since experimental errors in the case of minor components, although certainly less than one unit, become a considerable percentage error when referred to the amount of acid found. In spite of this we can say with certainty that the C_{20-22} acids tend to remain in the fat reserves when animals are starved, although a fair proportion of them are metabolized. The unsaturated acids of the C_{20-22} series are of especial importance in relation to the keeping quality and palatability of pig fats, and it was hoped in the course of the present experiments to obtain information as to the mobilization of these acids. It was shown [Hilditch *et al.* 1939] that these are cumulatively absorbed, at all events up to a certain point, from fish oil or fish meal in the diet. From Tables 3 and 4 it will be seen that these acids have not decreased during inanition (especially in the later stages) in the same proportion as the other components.

Again, whilst oleic acid is mobilized to a marked degree during the early stages of starvation, the structurally closely related linoleic acid is withdrawn from the depots to a less extent than any other acid over the corresponding period. It may be that this observation is connected with the special functions which, as first shown by Burr & Burr [1929], linoleic acid exercises in the animal diet. On the other hand, it is equally likely that the sparing of linoleic and of the unsaturated C_{20} and C_{22} acids during fat mobilization may be directly connected with the fact [Hilditch *et al.* 1939] that both of these acids arrive in pig depot fats by assimilation from dietary fats and are not synthesized by the animal; and that, correspondingly, they are less readily mobilized than those acids which are synthesized by the animal from carbohydrate.

To compare the depot fats of the pig and of fish is perhaps unprofitable, but the present results nevertheless resemble those of Lovern [1938] on flesh fats of

fasting herrings in that in both cases there is preferential utilization of unsaturated components; this effect, however, was little in evidence in the case of salmon during their fasting period [Lovern, 1934]. Similarities between our present data on pig depot fats and those of Longenecker [1939, 1, 2, 3] on rat depot fats are not very marked; the chief resemblances are the decrease in total C_{16} acids observed in two of the pig depots during the first 51 days of inanition. On the other hand, in one experiment with rats, Longenecker observed an increase in oleic acid content, and a decrease in linoleic acid content, during mobilization of fat from the rat depots.

The general results of this study are that, apart from relatively minor differences, there is no great evidence of selectivity in the mobilization of any one fatty acid component of the depot fats during starvation of the pig; the two most prominent subsidiary effects are the preferential removal of oleic acid during the later stages of inanition, and the definite reluctance in the earlier stages to mobilize those acids (linoleic and unsaturated C_{20-22} acids) which are derived from ingested dietary fats. Here, again, it should once more be emphasized that the problem of fat mobilization cannot be satisfactorily dealt with in terms of the fatty acids, but of the mixed triglycerides in which they are present. The most abundant glycerides in pig depot fats are palmitodioleins and oleopalmitostearins. If oleic acid is selectively desired, it therefore involves the concurrent removal of 1 mol. of palmitic acid for every 2 mol. of oleic acid, or of 1 mol. each of palmitic and stearic acid as well as oleic acid, in these respective glycerides. Similarly, as has already been pointed out, any of the minor component acids will only be represented once in the three acyl groups of any triglyceride and therefore, although an unsaturated C_{20} acid, for example, may not be so readily dealt with as oleic acid, a triglyceride molecule containing two oleic and one unsaturated C_{20} acyl group might well be attacked for the sake, as it were, of its oleic acid content. The problem of fat mobilization is, in fact, essentially one from which considerations of glyceride structure cannot be excluded: the individual mixed triglycerides present in the depot fat clearly have a profound bearing on the process.

Lastly, it should be borne in mind that, although the amounts of each acid combined in the depot fats have decreased during starvation of the pigs, concurrent synthesis of glycerides from non-fatty or fatty body constituents of the animal is not precluded, even though it is unlikely to take place to any great extent under the condition of fasting. The possibility cannot be excluded that, simultaneously with mobilization, building up (to a minor extent) of glycerides either from newly formed fatty acids or from acid fragments of partly metabolized mixed glycerides takes place. Well-known recent work by several investigators on the ingestion and mobilization of "labelled" fats has shown that both deposition and removal of fats in animal depots are relatively rapid and probably concurrent processes. The adipose tissues are now clearly recognized as dynamic, rather than static, reservoirs.

We again offer our thanks to Dr J. Hammond for his courtesy in providing the experimental material, to Drs E. H. Callow and C. H. Lea for their assistance in the extraction of the fats, and to the Department of Scientific and Industrial Research for permission to publish our results.

REFERENCES

Burr & Burr (1929). *J. biol. Chem.* **82**, 345.
Dowell (1920). *Ann. Rep. Oklahoma Agric. Exp. Sta.* **29**, 28.
Frank (1926). *Mschr. Kinderheilk.* **31**, 265.
Hilditch, Lea & Pedelty (1939). *Biochem. J.* **33**, 493.
Longenecker (1937). *J. Soc. chem. Ind., Lond.*, **56**, 199*T*.
—— (1939, 1). *J. biol. Chem.* **128**, 645.
—— (1939, 2). *J. biol. Chem.* **129**, 13.
—— (1939, 3). *J. biol. Chem.* **130**, 167.
Lovern (1934). *Biochem. J.* **28**, 1955.
—— (1938). *Biochem. J.* **32**, 676.
Mann (1936). *Fette u. Seifen*, **43**, 155.
Stefko (1928). *Biochem. Z.* **195**, 396.