Table 1. *Antibacterial activity of polyporenic acid C (as sodium salt)*

(Symbols: -, complete inhibition; ±, slight growth; +, moderate growth; ++, full growth; P, pellicle formation.)

Organism	Growth observed after	Dilutions 1 in								
		10,000	20,000	40,000	80,000	160,000	320,000	640,000	1,280,000	2,560,000
<i>Staph. aureus</i>	24 hr.	+	++	++	++	++	++	++	++	++
<i>B. coli</i>	24 hr.	-	++	++	++	++	++	++	++	++
<i>M. phlei</i>	72 hr.	-	-	-	-	-	-	-	-	++ P
<i>M. phlei</i>	7 days	+	++ P	.	.	.	.	.	.	.
<i>M. smegmatis</i>	72 hr.	-	-	-	-	-	-	-	++ P	++ P
<i>M. smegmatis</i>	7 days	++ P	.	.	.	.	.	.	.	.

Table 2. *Antibacterial activity of ungulinic acid (as disodium salt)*

(Symbols, as in Table 1.)

Organism	Growth observed after	Dilutions 1 in							
		2000	4000	8000	16,000	32,000	64,000	128,000	256,000
<i>Staph. aureus</i>	24 hr.	-	-	-	-	-	-	-	++
<i>B. coli</i>	24 hr.	++	++	++	++	++	++	++	++
<i>M. phlei</i>	77 hr.	-	-	-	± P	+ P	++ P	++ P	++ P
<i>M. phlei</i>	7 days	-	-	++ P	.	.	.	.	.

against *M. phlei* and *M. smegmatis*, using the medium of Dubos (1947), but the results were unsatisfactory, since the lower dilutions showed a turbidity which was undistinguishable from the growth of the organism. A point of minimum turbidity was noted however in each case, which may give some indication of the limiting dilution. This occurred with the triterpenoid acid at 1:16,000 and with ungulinic acid at 1:4000 for both species of Mycobacteria.

SUMMARY

1. The triterpenoid acid, polyporenic acid C, is a powerful antibacterial substance against Mycobacteria, but has little activity against *Staphylococcus aureus* and *Bacillus coli*. Even this high activity against Mycobacteria disappears on prolonged incubation.

2. Ungulinic acid is moderately active only against *Staph. aureus* of the organisms tested.

REFERENCE

Dubos, R. J. (1947). *Experientia, Basel*, 3, 45.

The Composition of the Depot Fats of a Pig Fed on a Diet Rich in Whale Oil

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The facility with which the pig can utilize dietary fat for the purpose of its own reserve fat has been recognized for many years. Thus, Ellis & Isbell (1926) showed that the depot fats of pigs fed respectively on soya beans and on ground nuts were markedly different from those of pigs fed on a normal (corn) diet low in fat and contained unusually large proportions of linoleic acid and minor

proportions of linolenic acid or of arachidic and higher saturated acids, which are present in the respective fatty oils of the soya bean or the ground nut. Similar results were observed by Ellis, Rothwell & Pool (1931) when cotton-seed oil was added to the normal diet of pigs. When a diet which included 14% of a fish (menhaden) oil was given to pigs, Brown (1931) observed that the pig body fat contained small but significant proportions of the unsaturated acids of the C₂₀ and C₂₂ series which are characteristic components of the fish oil.

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An exceptionally favourable opportunity to study this matter in further detail recently occurred when specimens of the adipose tissues of a pig which had been reared on a diet exceptionally rich in whale oil at the Animal Research Station, Cambridge, were placed at our disposal by the Director of Food Investigation, Dr F. Kidd, F.R.S. The animal had been fed from weaning on a diet which contained 50 % of crude whale oil and in the final stages of fattening was receiving 4 lb. of whale oil per day. At slaughter (at 198 days) it weighed over 200 lb., its rate of growth being normal and its condition apparently fully healthy. The fat depots after slaughter were found to have a yellowish brown coloration and to present certain abnormal features which led Mr J. K. Walley (who was carrying out the investigation) to consult us. The average unsaturation (iodine value) of the depot fats was abnormally high and the specimens appeared to merit a quantitative examination of their component acids and glycerides, which we readily agreed to undertake and the results of which are here recorded.

METHODS AND RESULTS

Specimens of the perinephric, inner back and outer back adipose tissues sent to us were extracted exhaustively with acetone. The matter extracted by acetone was taken up in light petroleum (b.p. 40–60°), and the solution filtered from insoluble matter, after which the lipid matter from each tissue was separately recovered and found to have the general characteristics shown in Table 1.

Table 1. *Analytical characteristics of the pig depot fats*

	Source of fat		
	Perinephric	Inner back	Outer back
Iodine value	83.7	85.7	86.5
Saponification equivalent	286.4	287.8	286.0
Free fatty acid (as oleic, %)	0.6	0.9	0.4
Unsaponifiable matter (%)	1.2	0.2	0.8
Peroxide value	0.3	4.8	5.1

Component fatty acids of the pig fats

The mixed acids from about 120 g. of each fat were examined quantitatively by preliminary resolution into three (or four) groups by crystallization, first from acetone at –60°, the deposited acids (in one instance after a recrystallization from acetone at –60°) being then crystallized from ether at –30°. The methods used for the fractional distillation of the methyl esters of each group of acids and for the analytical (including spectrophotometric) examination of the ester fractions, were those described (Gupta & Hilditch, 1951). In order, however, to illustrate the typically 'marine animal' nature of the acids left in solution in acetone at –60°, the ester fractionation data for the groups of acids from all three depot fats are recorded in Table 3. The results of the solvent separations of the mixed fatty acids are summarized in Table 2.

Table 2. *Low temperature crystallization of the mixed acids from each pig depot fat*

Solubility characteristics of fraction	Weight		Iodine value
	(g.)	(% of total)	
Perinephric fat			
<i>A.</i> Insoluble in ether at -30°	39.0	35.5	3.1
<i>B.</i> Soluble in ether at -30°	34.4	31.3	77.7
<i>C.</i> Soluble in acetone at -60°	36.5	33.2	177.1
Inner back fat			
<i>A.</i> Insoluble in ether at -30°	31.6	30.2	2.8
<i>B.</i> Soluble in ether at -30°	26.9	25.7	78.0
<i>C.</i> Soluble in acetone at -60°	45.9	44.1	148.3
Outer back fat			
<i>A.</i> Insoluble in ether at -30°	29.9	27.9	6.2
<i>B.</i> Soluble in ether at -30°	42.7	39.9	83.9
<i>C.</i> Insoluble in acetone at -60° (recrystallization)	9.2	8.6	148.2
<i>D.</i> Insoluble in acetone at -60° (first crystallization)	25.3	23.6	175.8

The composition of each ester fraction was then calculated by methods described (Hilditch, 1947, pp. 498–510; Gupta & Hilditch, 1951), and therefrom the percentage composition (by wt.) of each group of acids from each fat. These compositions and the consequent composition of the total fatty acids in each of the three depot fats are shown in Table 4.

Glyceride composition of the pig perinephric fat

The figures in Table 4 show clearly that all three depot fats of the pig which had received a diet rich in whale oil contained considerable amounts of the highly unsaturated acids characteristic of whale oil and, indeed, that these were of much the same average unsaturation as those in a typical whale oil (cf. Table 6). It was therefore decided to see whether, by suitable crystallization procedure, one of the fats could be separated roughly into fractions which would resemble respectively whale oil and the type of fat which the pig is known to elaborate from a non-fatty diet. It is known (Hilditch & Maddison, 1948) that all but about 20 % of the (more saturated) glycerides in Antarctic whale oil remain in solution in acetone at –40°, whereas only a very small percentage of pig depot fat glycerides from animals on a diet low in fat are soluble in acetone at this temperature.

A quantity of the pig perinephric fat was therefore crystallized from a 10 % solution in acetone at –40°, the deposited glycerides being recrystallized under the same conditions and the procedure repeated until only a very small amount of glycerides remained in solution. In this way the following fractions were successively obtained:

	Weight (g.)	Iodine value
First crystallization	38.7	163.5
Second crystallization	15.7	136.0
Third crystallization	7.7	126.0
Fourth crystallization	2.6	122.0
Fifth crystallization	2.5	112.4
Glycerides deposited (fifth crystallization)	191.9	59.7

Table 3. *Fractionation data obtained with esters of fatty acids from depot fats of pig*

(The solubility characteristics of fractions A, B, C and D are given in Table 2.)

Fraction	Weight (g.)	Sapon. equiv.	Iodine value	Fraction	Weight (g.)	Sapon. equiv.	Iodine value
Perinephric fat				Inner back fat			
Methyl esters of acids A:				Methyl esters of acids A:			
A1	2.17	255.0	0.4	A1	2.09	265.9	1.2
A2	3.81	265.9	0.6	A2	3.19	270.4	1.2
A3	4.40	269.6	0.4	A3	3.67	271.0	1.5
A4	4.18	270.1	0.7	A4	4.08	273.3	1.7
A5	4.24	271.5	0.9	A5	4.28	275.7	2.5
A6	5.47	281.3	5.1	A6	4.09	278.7	3.0
A7	8.25	294.9	6.4	A7	3.61	286.9	4.3
A8	2.61	297.0	6.5	A8	4.22	288.2	4.8
A9	2.34	300.4*	8.2	A9	2.91	294.7†	8.2
Total	37.47	—	—	Total	32.14	—	—
Methyl esters of acids B:				Methyl esters of acids B:			
B1	1.40	247.8	10.8	B1	2.46	268.1	38.5
B2	1.83	268.5	33.8	B2	2.94	287.1	67.4
B3	3.35	286.7	66.1	B3	3.66	288.0	69.2
B4	3.03	291.5	75.5	B4	4.31	292.7	79.0
B5	3.39	293.2	75.8	B5	3.75	295.8	80.2
B6	2.99	293.6	79.6	B6	3.29	295.7	81.6
B7	3.99	294.8	79.5	B7	4.27	296.8	83.5
B8	3.02	295.3	80.8	B8	3.00	300.5†	87.8
B9	3.27	295.4	81.5	Total	27.58	—	—
B10	3.16	296.8	82.7				
B11	2.63	303.9*	87.1				
Total	32.06	—	—				
Methyl esters of acids C:				Methyl esters of acids C:			
C1	1.14	205.6	47.6	C1	1.95	242.8	38.5
C2	2.48	261.3	86.0	C2	4.11	260.7	91.2
C3	3.90	273.5	102.0	C3	3.24	277.2	100.8
C4	2.98	288.7	119.3	C4	4.82	288.9	108.1
C5	3.40	287.6	120.0	C5	4.65	291.7	111.0
C6	3.27	289.7	126.8	C6	5.96	295.6	110.5
C7	3.78	293.4	133.6	C7	5.38	296.0	114.0
C8	3.08	301.4	185.0	C8	4.47	298.0	121.9
C9	3.19	303.2	193.4	C9	4.15	301.4	149.4
C10	3.45	307.2	219.3	C10	4.15	314.5	239.4
C11	3.71	325.4	334.0	C11	3.88	333.1†	300.8
C12	2.19	359.8*	257.0	Total	46.76	—	—
Total	36.57	—	—				
Outer back fat							
Methyl esters of acids A:				Methyl esters of acids C:			
A1	3.24	254.0	1.2	C1	1.63	267.3†	83.6
A2	2.50	269.4	1.0	C2	1.80	282.0	111.5
A3	3.03	270.0	2.0	C3	1.90	291.7	123.1
A4	3.67	274.3	4.4	C4	1.37	299.2	153.7
A5	4.50	275.9	5.2	C5	1.24	306.4	178.7
A6	2.78	275.2	5.6	C6	1.06	351.3†	232.0
A7	3.15	285.3	10.3	Total	9.00	—	—
A8	3.48	291.7	11.1				
A9	3.44	297.8†	14.8				
Total	29.79	—	—				
Methyl esters of acids B:				Methyl esters of acids D:			
B1	1.78	238.4	30.5	D1	1.22	249.4†	67.8
B2	2.42	274.3	54.4	D2	2.12	267.3	90.8
B3	3.53	286.1	65.6	D3	2.27	268.9	108.3
B4	2.93	287.1	67.7	D4	2.30	284.5	128.7
B5	4.08	296.4	82.8	D5	2.23	286.0	128.6
B6	4.17	296.2	84.4	D6	2.79	289.5	134.3
B7	5.73	297.8	83.8	D7	3.06	293.8	143.7
B8	5.57	298.6	85.3	D8	2.17	296.6	168.2
B9	4.30	300.4	85.2	D9	2.57	307.4	212.5
B10	4.76	308.4†	87.9	D10	2.14	319.2	272.8
B11	2.69	317.4†	101.9	D11	2.55	328.3†	303.9
Total	41.96	—	—	Total	25.42	—	—

* Equivalents of esters (freed from unsaponifiable matter): A9, 297.6; B11, 297.1; C12, 333.2.

† Equivalents of esters (freed from unsaponifiable matter): A9, 288.5; B8, 300.5; C11, 329.2.

‡ Equivalents of esters (freed from unsaponifiable matter): A9, 294.1; B10, 301.4; B11, 308.0; C1, 263.1; C6, 342.5; D1, 248.3; D11, 328.3.

Table 4. *Component acids in groups A, B, C (D), and in the whole pig depot fats*

(Separation into fractions A, B, C and D is described in Table 2.)

Acid	A	B	C	D	Fatty acids in the whole fat	
	(%)*	(%)*	(%)*	(%)*	(%, w/w)	(%, by mol.)
Perinephric fat						
	(35.5%)†	(31.3%)†	(33.2%)†	—		
Myristic acid	4.5	3.1	1.9	—	3.3	4.1
Palmitic acid	55.3	9.7	4.7	—	24.2	25.8
Stearic acid	36.2	2.0	—	—	13.5	12.9
Unsaturated C ₁₄	—	0.3	2.7	—	1.0	1.2
Unsaturated C ₁₆	0.3	3.4	15.1	—	6.2	6.7
Unsaturated C ₁₈	3.7	80.7	48.6	—	42.8	41.4
Unsaturated C ₂₀	—	0.6	20.3	—	7.0	6.2
Unsaturated C ₂₂	—	—	6.3	—	2.0	1.7
Unsaponifiable	Trace	0.2	0.4	—	—	—
Mean unsaturation† of:						
C ₁₄ acids	—	-2.0	-2.0	—		-2.0
C ₁₆ acids	-2.0	-2.0	-2.1	—		-2.1
C ₁₈ acids	-2.0	-2.0	-3.3	—		-2.5
C ₂₀ acids	—	-3.0	-7.3	—		-7.2
C ₂₂ acids	—	—	-10.3	—		-10.3
Inner back fat						
	(30.2%)†	(25.7%)†	(44.1%)†	—		
Myristic acid	0.9	0.3	2.8	—	1.6	1.9
Palmitic acid	62.2	11.2	2.4	—	22.7	24.3
Stearic acid	33.1	2.5	—	—	10.6	10.3
Unsaturated C ₁₄	—	—	3.2	—	1.4	1.7
Unsaturated C ₁₆	0.4	5.6	13.2	—	7.4	8.0
Unsaturated C ₁₈	3.2	78.0	59.4	—	47.3	45.8
Unsaturated C ₂₀	—	2.4	15.7	—	7.6	6.8
Unsaturated C ₂₂	—	—	3.2	—	1.4	1.2
Unsaponifiable	0.2	—	0.1	—	—	—
Mean unsaturation† of:						
C ₁₄ acids	—	—	-2.0	—		-2.0
C ₁₆ acids	-2.0	-2.0	-2.1	—		-2.1
C ₁₈ acids	-2.0	-2.0	-2.7	—		-2.4
C ₂₀ acids	—	-3.0	-6.5	—		-6.2
C ₂₂ acids	—	—	-8.5	—		-8.5
Outer back fat						
	(27.9%)†	(39.9%)†	(8.6%)†	(23.6%)†		
Myristic acid	5.9	3.0	2.9	1.6	3.5	4.2
Palmitic acid	58.5	6.1	3.0	2.0	19.5	20.8
Stearic acid	28.4	0.8	—	—	8.2	7.9
Unsaturated C ₁₄	—	1.2	—	2.0	0.9	1.2
Unsaturated C ₁₆	1.8	3.5	23.4	24.1	9.7	10.4
Unsaturated C ₁₈	5.3	75.4	49.3	45.5	46.7	45.4
Unsaturated C ₂₀	—	9.5	11.2	18.6	9.2	8.2
Unsaturated C ₂₂	—	—	9.5	6.2	2.3	1.9
Unsaponifiable	0.1	0.5	0.7	Trace	—	—
Mean unsaturation† of:						
C ₁₄ acids	—	-2.0	—	-2.0		-2.0
C ₁₆ acids	-2.0	-2.0	-2.1	-2.1		-2.1
C ₁₈ acids	-2.0	-2.0	-3.1	-3.4		-2.4
C ₂₀ acids	—	-3.0	-5.2	-6.8		-5.1
C ₂₂ acids	—	—	-6.4	-8.8		-7.9

* Component acids as % (w/w) of group.

† Groups as % (w/w) of total acids.

‡ Mean unsaturation expressed as number of hydrogen atoms necessary to convert 1 mol. of acid to the saturated state (Hilditch, 1947, p. 23).

The portions left in solution were mixed and the fatty acids in the two groups so obtained were analysed in detail by the methods described above. There were thus obtained:

	Weight		Iodine value
	(g.)	(% of total)	
Glycerides insoluble in acetone at -40°	191.9	74.1	59.7
Glycerides soluble in acetone at -40°	67.2	25.9	149.6

The final results of the component acid analyses of each group are summarized in Table 5.

Table 5. *Component fatty acids (% w/w) of the pig perinephric glycerides separated by crystallization from acetone at -40°*

Acid	Insoluble in acetone at -40°	Soluble in acetone at -40°
Myristic acid	2.1	1.5
Palmitic acid	25.7	14.3
Stearic acid	16.5	1.4
Unsaturated C_{14}	0.3	0.8
Unsaturated C_{16}	5.4	11.1
Unsaturated C_{18}	43.5	44.3
Unsaturated C_{20}	6.5	21.2
Unsaturated C_{22}	—	5.4
Mean unsaturation of:		
C_{14} acids	-2.0	-2.0
C_{16} acids	-2.1	-2.1
C_{18} acids	-2.3	-2.9
C_{20} acids	-6.4	-6.8
C_{22} acids	—	-10.3

Comparison of the figures in Table 5 with those of a typical perinephric fat from a pig fed on a low-fat diet and of a typical Antarctic whale oil (Table 6) shows that the fat under investigation had been largely resolved by the physical process of crystallization into pig synthetic fat and the assimilated dietary fat.

DISCUSSION

The figures in Table 4 not only demonstrate the marked extent to which the component acids of the three depot fats differ from those of a pig fed on a diet low in fat, but suggest that the component acids of whale oil are present in significant amounts and that, moreover, the composition of these abnormal pig depot fats might be explained on an additive basis: they appear, *prima facie*, to be a mixture of typical pig depot fat (as synthesized from carbohydrate) with more or less unaltered whale oil. This tentative conclusion is reinforced by more detailed examination of the fatty acid compositions observed in each of the three fats.

Fortunately, it is well established that the respective compositions of perinephric, inner and outer back fats from pigs fed on diets low in fat are remarkably uniform (Ellis & Isbell, 1926; Ellis & Zeller, 1930; Ellis *et al.* 1931; Hilditch, Lea & Pedelty, 1939; Hilditch & Pedelty, 1940), and consequently figures for such biosynthesized fats are available. In addition, although the specimen of Antarctic whale oil fed to the pigs was not analysed in detail, the component fatty acids of Antarctic whale oils have been found to vary but little over a range of several seasons (Armstrong & Allan, 1924; Tveraaen, 1935; Hilditch & Maddison, 1948). Typical figures for the component acids of depot fats of pigs fed on a low-fat diet (Hilditch & Pedelty, 1940) and of Antarctic whale oil (Hilditch & Maddison, 1948) are given in Table 6.

The palmitic acid contents of all the fats in Table 6 are highly characteristic. In a mixture of pig fat and whale oil, therefore, the approximate proportions of each of these fatty oils could be deduced from the percentage of palmitic acid in the total fatty acids of the mixture (Table 7).

The fatty acid compositions of mixtures of the respective depot fats (Table 6) with Antarctic whale

Table 6. *Component acids (% w/w) of depot fats of pigs (on low-fat diet) and component acids of Antarctic whale oil*

Acid	Pig depot fats			Antarctic whale oil
	Perinephric	Inner back	Outer back	
Myristic acid	0.9	0.9	1.0	9.2
Palmitic acid	29.3	27.5	26.5	15.6
Stearic acid	17.4	15.1	12.8	2.5
Unsaturated C_{14}	0.3	0.2	0.2	2.5
Unsaturated C_{16}	1.8	1.7	1.9	13.9
Unsaturated C_{18}	48.4	51.5	54.7	37.2
Unsaturated C_{20}	1.9	3.1	2.9	12.0
Unsaturated C_{22}				7.1
Mean unsaturation of:				
C_{14} acids	-2.0	-2.0	-2.0	-2.0
C_{16} acids	-2.0	-2.0	-2.0	-2.1
C_{18} acids	-2.3	-2.3	-2.3	-2.4
C_{20} acids	-5.0	-5.0	-5.0	-7.1
C_{22} acids				-9.4

oil in the proportions shown in Table 7 have been calculated and are compared in Table 8 with the results actually found for the three pig depot fats (Table 4). The close similarity between the 'calculated' figures and those found for each individual acid (including the mean unsaturation of the C₂₀

and C₂₂ acids present) appears to offer conclusive proof that the whale oil has been deposited in a purely additive manner alongside the fat synthesized by the pig. Moreover, in the outer layers of the back fat, which are invariably softer and more unsaturated than the inner layers or the perinephric fat, the increase in unsaturation appears to be produced by a marked increase in the proportion of deposited whale oil.

The figures in Table 8 can hardly be interpreted on any other basis than that whale oil, substantially unchanged, has passed along with the fat synthesized by the pig itself into the fat depots of the animal and, further, that the typical differences in unsaturation and consistency of fats in the different depots have been attained by variation in the pro-

Table 7. *Calculated proportions of (biosynthesized) pig fat and whale oil (calculated from the percentage of palmitic acid in the three depot fats)*

	Palmitic acid in depot fatty acids (%)	Calculated proportions of	
		True pig fat (%)	Whale oil (%)
Perinephric fat	24.2	62	38
Inner back fat	22.7	60	40
Outer back fat	19.5	36	64

Table 8. *Comparison of observed and 'calculated' compositions of the pig depot fats.*
(Component fatty acids, % w/w)

(For details of calculation see text and Tables 6 and 7.)

Acid	Source of fat					
	Perinephric		Inner back		Outer back	
	Found	'Calculated' (62% pig: 38% whale)	Found	'Calculated' (60% pig: 40% whale)	Found	'Calculated' (36% pig: 64% whale)
Myristic acid	3.3	4.1	1.6	4.2	3.5	6.2
Palmitic acid	24.2	(24.2)	22.7	(22.7)	19.5	(19.5)
Stearic acid	13.5	11.7	10.6	10.1	8.2	6.2
Unsaturated C ₁₄	1.0	1.1	1.4	1.1	0.9	1.7
Unsaturated C ₁₆	6.2	6.4	7.4	6.6	9.7	9.6
Unsaturated C ₁₈	42.8	44.1	47.3	45.8	46.7	43.5
Unsaturated C ₂₀	7.0	5.3	7.6	6.0	9.2	8.4
Unsaturated C ₂₂	2.0	3.1	1.4	3.5	2.3	4.9
Mean unsaturation of:						
C ₁₄ acids	-2.0	-2.0	-2.0	-2.0	-2.0	-2.0
C ₁₆ acids	-2.1	-2.0	-2.1	-2.0	-2.1	-2.1
C ₁₈ acids	-2.5	-2.3	-2.4	-2.3	-2.4	-2.4
C ₂₀ acids	-7.2	-7.0	-6.2	-7.0	-5.1	-7.0
C ₂₂ acids	-10.3	-9.0	-8.5	-9.0	-7.9	-9.0

Table 9. *Comparison of the solvent-segregated perinephric fat with normal pig perinephric fat and whale oil.*
(Component fatty acids, % w/w)

Acid	Present perinephric fat (Table 5)			
	Typical pig perinephric fat	Insoluble portion	Soluble portion	Antarctic whale oil
Myristic acid	0.9	2.1	1.5	9.2
Palmitic acid	29.3	25.7	14.3	15.6
Stearic acid	17.4	16.5	1.4	2.5
Unsaturated C ₁₄	0.3	0.3	0.8	2.5
Unsaturated C ₁₆	1.8	5.4	11.1	13.9
Unsaturated C ₁₈	48.4	43.5	44.3	37.2
Unsaturated C ₂₀	1.9	6.5	21.2	12.0
Unsaturated C ₂₂		—	5.4	7.1
Mean unsaturation of:				
C ₁₄ acids	-2.0	-2.0	-2.0	-2.0
C ₁₆ acids	-2.0	-2.1	-2.1	-2.1
C ₁₈ acids	-2.3	-2.3	-2.9	-2.4
C ₂₀ acids	-5.0	-6.4	-6.8	-7.1
C ₂₂ acids		—	-10.3	-9.4

portions of whale oil and synthesized pig fat laid down. This conclusion is reinforced by the resolution of the perinephric fat by the physical process of crystallization under appropriate conditions into 26 % of a fatty oil not very different from whale oil and 74 % of a fat closely resembling pig perinephric fat as synthesized in the animal (Table 9).

Under the conditions of crystallization some 20 % of the more saturated glycerides of any whale oil present would have been left in the insoluble fraction, so that the 26 % of soluble matter probably represents about 33 % of whale oil originally present. Exact reproduction respectively of a typical 'synthesized' pig fat or of whale oil by either segregate is therefore not to be expected, but the approach towards each oil is somewhat striking, especially in the palmitic and stearic acid contents of each portion. The presence of a small proportion of characteristic whale oil acyl groups in the insoluble portion is indicated by the larger proportions of unsaturated C_{16} and C_{20} acids as compared with a typical pig perinephric fat.

Since dietary fat is largely assimilated through the lymphatic system before it enters the blood stream, it is perhaps not unnatural that the depot fats of a pig fed heavily on whale oil should exhibit the compositions disclosed in the course of this study. Yet we believe that the conditions governing the deposition of dietary and synthesized fats have not previously been depicted so clearly as in the present instance, and we are glad to have had the opportunity to examine these especially informative specimens. It is, moreover, somewhat remarkable that the highly reactive unsaturated groups in the marine animal fat have undergone no perceptible alteration during their passage through the animal: nor, indeed, is there any indication of chemical interchange between the acyl groups of the fat synthesized in the pig and the whale glycerides. Had this taken place to any appreciable extent the physical resolution of the fat by solvent segregation could not have given the results which were in fact obtained. At first sight it might also be supposed that the present study throws further light on the processes involved in the passage of dietary fat into the lymphatic system, but this is not so. Since the whale oil was ingested in massive proportions, it would represent practically all the fat which was absorbed as such: consequently, its constituent glycerides would be substantially the same whether they were hydrolysed and resynthesized during passages from the intestine into the lymphatics, or whether they were translocated as such without hydrolysis. It would seem, however, that with a

suitably modified fatty diet it should be possible to obtain some more conclusive evidence in this direction, and it is hoped that one of us may be able to undertake further studies to this end in the near future.

SUMMARY

1. The component acids present in the perinephric, inner back and outer back fats of a pig which had been fed heavily on whale oil have been examined. The results show that considerable proportions of the characteristic whale oil fatty acids had passed into the fat depots, especially in the case of the outer back fat.

2. Crystallization of the perinephric fat from acetone at -40° separated it into 26 % of a soluble fraction which approximated in composition to whale oil, and left 74 % of insoluble material, the composition of which closely resembled a normal pig perinephric fat except for the presence of somewhat more unsaturated acids of the C_{16} and C_{20} series than are usually found in the latter.

3. The depot fats from the animal whose diet had included 50 % of whale oil therefore consist of a mixture of whale oil with the typical fat synthesized by the pig from non-fatty food. The compositions of the individual component acids in each of the three fats, when calculated on the assumption that they were mixtures of typical 'synthesized' depot fats with whale oil in appropriate proportions, agreed closely in most cases with those determined experimentally.

4. It was noticeable that the high degree of unsaturation of the acids of the C_{20} and C_{22} series in whale oil was reproduced in the corresponding fatty acids of the deposited fat. This emphasizes the manner in which the whale oil glycerides had been assimilated and deposited in the depots without any chemical alteration.

5. The greater unsaturation (leading to softer consistency) of the fat in the outer layers of the back adipose tissue was attained by a relatively higher proportion of deposited whale oil to typical synthesized pig fat than in the inner layers or perinephric fat.

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Observations on the Determination of the Specific Activity of the Inorganic Phosphate Fraction of Trichloroacetic Acid Extracts of Liver

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The determination of the specific activity (S.A.) of inorganic phosphorus* present in a tissue extract involves its quantitative separation from organophosphates and the subsequent measurement of the radioactivity. Measurement of inorganic P is made directly on another sample. The method generally employed for separation involves a precipitation of the inorganic P either as a molybdenum salt, or as the ammonium magnesium salt, but the latter technique is generally preferred because of the high absorbing power of molybdenum for β -radiation (Hevesy, 1948). Because of the rather great sensitivity of the methods employed for the measurement of the radioactivity, careful attention to the conditions of precipitation is necessary. Prime among the requirements is that ample time should be allowed for completeness of precipitation and that sufficient carrier P be present. Generally the ammoniacal solution, from which inorganic P is being precipitated, is kept overnight at 0–5° to ensure complete precipitation.

There is some doubt about the amount of carrier P which should be added. The literature shows that most workers add carrier P, but it is rare to find any reference to the amount which has, in fact, been added. Theoretically, since it is a question of measuring only ^{32}P , the amount of non-radioactive P which is present is of no consequence, provided

* In this paper the term 'inorganic phosphate' refers to that form of phosphorus which can be precipitated as magnesium ammonium phosphate and which will react directly, in acid solution, with ammonium molybdate to form a reducible phosphomolybdate.

that sufficient has been added to ensure complete precipitation of ^{32}P , and that correction factors are applied should the precipitate being counted exceed a limiting thickness. From the practical viewpoint, however, the amount of carrier P which is present is of considerable importance and it is the purpose of this communication to show that serious errors are introduced into the determination of the S.A. by the use of conventional techniques. A preliminary report of this work has already been published (Ennor & Rosenberg, 1951). A method will also be described, by means of which the accurate and rapid determination of the S.A. of inorganic P may be made in presence of labile organophosphates.

A recent publication by Ennor & Stocken (1950) has given an account of a method for the determination of inorganic P in the presence of acid-molybdate-labile organophosphates. Briefly, this method, which is a modification of that described by Berenblum & Chain (1938), involves the combination of molybdic acid and inorganic P in an acid aqueous phase and the extraction of the resultant phosphomolybdic acid with *isobutanol*. Both reaction and extraction are accomplished in 10 sec. and thus only the P released in this time interval from any acid-molybdate-labile organophosphates present adds to the inorganic P. It was shown that the P so measured is subject to an error equal to only 1% of the amount of P present as phosphocreatine, this being the most labile P compound present in most tissue extracts. It occurred to us that this method would be of value in experiments involving ^{32}P , since the