CX. THE BODY FATS OF THE HEN.

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(*Received April 12th, 1934.*)

THE examination of bird body fats has hitherto been restricted to separation of the saturated acids present by means of their lead salts (Twitchell) or by oxidation (Bertram). Bömer and Merten [1922] state that the acids present in a sample of goose fat were palmitic (21·8 %), stearic (3·9 %) and oleic (74·3 %), while Grossfeld [1930; 1931] reports for the same fat lower volatile acids (0·2 %), palmitic (20·9 %), stearic (10·6 %), oleic (49·0 %) and linoleic (19·3 %) acids[1]. The latter author also gives two similar component fatty acid analyses for chicken fat as follows: lower volatile acids (0·1 %), palmitic (18·4, 19·3 %), stearic (8·9, 7·5 %), oleic (54·7, 55·4 %) and linoleic (17·9, 17·8 %) acids. Grossfeld pointed out however that the percentage of palmitic acid, calculated from the saponification value of the total mixed fatty acids, was about 10 units higher than that calculated from the molecular weight of the separated "solid" fatty acids and attributed this to the presence of fatty acids of lower molecular weight than that of palmitic acid.

The work about to be recorded proves that this discrepancy is not due in hen body fats to the presence of lower saturated fatty acids, but to that of about 7–8 % of a palmitoleic acid. Indications of this or a similar acid were recently reported by Banks *et al.* [1933] in rat body fats, although lack of material prevented decisive evidence being obtained.

The primary objectives of our experiments were to ascertain the general nature of a bird fat and to compare the depot fats from different parts of the body. We were fortunate in having the co-operation of Dr E. M. Cruickshank, who not only supervised the rearing of the fowls, but was also good enough to separate for us the fatty tissues from the abdominal layers, gizzards[2] and necks of the birds. We wish to express our great appreciation of the help thus afforded to us by Mr Halnan and Dr Cruickshank of the Cambridge School of Agriculture.

The specimens of the three body fats which we examined in the first instance were from 12 Light Sussex hens, 2 years old, whose diet had included 7 % of fish meal containing about 2·5 % of fish fat. The results of analyses by the ester fractionation method showed, as stated, that palmitoleic acid and also small amounts of unsaturated acids of the C_{20} and C_{22} series were present; and a new series of fats from 24 Light Sussex hens (which had received no fish meal), 7 months old, was therefore studied, in order to ascertain whether these acids

[1] The figures given here are the percentages of each acid on the *mixed component fatty acids*, and not (as in the original memoirs) those in the whole fats.

[2] The "gizzard fat" consisted of the layer of fat adherent to the gizzard together with the fat of the mesenteric membrane and any which was packed around the kidneys. Roughly speaking therefore this fat might perhaps be compared with the leaf and inner back fats of the pig, whilst the abdominal layer is in some respects more akin to the outer layers of back fat of the pig.

originated from those present in the fish meal fat. The results however were closely similar to those for the original group, and it is inferred that these acids are normal components of hen depot fats.

Since the diet of the second batch of 24 hens did not contain fish meal, it is perhaps preferable to deal with them first in the present paper. The diet of each group of birds, from 6 weeks after hatching onwards[1], was made up of mash (bran, maize and Sussex ground oats 20 parts each and sharps 40 parts) and grain (maize and wheat). For the group of 24 hens, 10 % of extracted soya bean meal and 2 % of mineral mixture were added to the mash, whilst to the mash given to the group of 12 hens there were added 7 % each of extracted soya bean meal and fish meal (containing 2·4 % fat) and 3 % of mineral mixture.

The average analyses of the rations were as follows:

	Moisture %	Ether extract %	Protein %	Carbohydrate %	Fibre %	Ash %
24 hens (fish meal-free)	12·4	4·0	16·1	55·3	6·5	5·7
12 hens (with fish meal)	12·5	3·8	14·1	57·9	6·6	5·1

The fats were extracted from the tissues by boiling with acetone; at the ordinary temperature they consisted chiefly of deep-yellow liquids from which a certain quantity of white solid glycerides deposited. Their general characteristics are given in Table I.

Table I. *General characteristics of hen fats.*

	Yield of fat from adipose tissue %	Extracted fat			Mixed acids setting-point ° C.
		Sap. equiv.	I.V.	Acid value	
From 24 hens (fish meal-free diet):					
Abdominal	97·5	285·5	80·1	0·7	34·5
Gizzard	96·9	286·0	78·8	1·7	35·1
Neck	96·0	285·9	79·8	1·4	34·6
From 12 hens (7 % fish meal in diet):					
Abdominal	96·4	286·3	78·5	0·7	36·2
Gizzard	95·8	284·5	79·7	5·6	36·5
Neck	88·7	284·8	77·4	1·2	36·5

The component acids present in each fat were separated by means of lead salts into "solid" and "liquid" acids, after which the methyl esters of each of these groups of acids were fractionally distilled in a vacuum. Analyses of the esters of the "solid" acids furnished quite normal results, but those of the esters of the "liquid" acids showed two unusual features. In the first place, the penultimate and the residual ester fractions in this group possessed analytical characteristics which indicated the presence of small amounts of unsaturated acids of the C_{20} and C_{22} series; the proportions present were approximately calculated as described by Banks and Hilditch [1932], who encountered a similar instance in the case of sow body fats. In the second place, the saponification equivalents of the most volatile fractions of the "liquid" esters (obtained by refractionation of the lowest-boiling primary fraction) were unusually low, and when the unsaturated material present therein was calculated on the assumption

[1] During the first 6 weeks the birds were in confinement and their ration included minute amounts of olive oil containing calciferol (0·001 ml. per bird daily); after 6 weeks the birds had access to free range.

Table II. *Complete analytical data for the abdominal and gizzard fats from the group of 24 hens.*

(i) Lead salt separation of mixed fatty acids.

	Abdominal layer fat		Gizzard fat	
	g.	%	g.	%
"Solid" acids S	91·1	31·8	89·6	31·1
"Liquid" acids L	195·0	68·2	198·6	68·9

(ii) Fractional distillation of the methyl esters of the "solid" acids (S).

No.	g.	Sap. equiv.	I.V.	g.	Sap. equiv.	I.V.
S 1	4·16	268·9	1·4	3·59	269·5	1·3
S 2	4·71	269·9	1·4	5·87	269·8	1·4
S 3	7·93	270·8	1·4	8·02	270·4	1·6
S 4	11·77	272·3	1·8	9·75	272·0	1·8
S 5	10·86	273·5	2·3	15·53	272·5	2·0
S 6	11·62	274·9	3·0	14·76	276·1	3·2
S 7	11·89	278·9	4·2	14·63	280·9	4·8
S 8	10·37	288·5	6·6	6·01	289·2	6·6
S 9	3·46	297·6	10·8	6·22	296·9	9·5
	76·77			84·38		

(iii) Fractional distillation of the methyl esters of the "liquid" acids (L).

No.	g.	Sap. equiv.	I.V.	g.	Sap. equiv.	I.V.
L 1	29·53	286·1	104·8	33·92	284·4	104·1
L 2	11·59	291·6	112·8	9·94	291·7	116·5
L 3	12·13	293·1	114·0	10·86	292·9	117·4
L 4	10·99	295·1	115·4	9·89	293·6	117·6
L 5	11·47	295·4	116·0	11·94	293·6	119·6
L 6	12·18	296·5	114·5	6·87	294·8	118·1
L 7	4·65	312·7	114·8	7·23	308·6	123·2
	92·54	(297·8)*	(116·0)*	90·65	(297·1)*	(119·9)*

(iv) Determination of saturated esters in fractions L 1.

Wt. oxidised (g.)	25·79	30·15
Wt. of saturated esters recovered (g.)	2·24	2·92
I.V. of saturated esters recovered	1·0	3·9
Sap. equiv. of saturated esters recovered	267·7	269·9

(v) Fractional distillation of the hydrogenated methyl esters of the "liquid" acids (HL).

No.	g.	Sap. equiv.	I.V.	g.	Sap. equiv.	I.V.
HL 1	2·83	279·0	—	4·86	280·2	1·2
HL 2	3·02	283·1	—	9·65	287·5	1·5
HL 3	6·10	287·2	—	12·00	290·6	1·9
HL 4	9·23	290·7	—	13·40	293·9	2·0
HL 5	11·77	293·3	—	14·33	295·8	2·1
HL 6	11·47	295·9	—	14·89	297·0	2·1
HL 7	13·88	296·3	—	9·18	298·8	1·9
HL 8	13·66	297·6	—	7·58	313·6	10·1
HL 9	7·02	299·0	0·3	—	—	—
HL 10	5·42	306·9	1·8	—	—	—
	84·40	(301·2)*	(1·7)*	85·89	(299·9)*	(6·9)*

* Saponification equivalents and iodine values of residual esters freed from unsaponifiable material.

that only unsaturated esters of C_{18} acids were present, the consequent equivalent of the saturated esters indicated the presence of methyl laurate or of esters of even lower molecular weight [*cf.* Grossfeld, 1930; 1931]. Careful qualitative examination of the acids present in these most volatile fractions showed on the other hand that the saturated acid actually present was mainly palmitic, probably accompanied by minor amounts of myristic acid; and no trace could be detected of acids of lower molecular weight than myristic acid.

The methyl esters of the "liquid" fatty acids were therefore divided into two halves, one of which was fractionally distilled without further treatment, while the other was similarly treated after converting it practically completely into saturated esters by hydrogenation in presence of a nickel catalyst at 170–180°. In the direct fractionation of the "liquid" esters, the first fraction collected (which was usually about one-third of the material distilled) was not submitted to further refractionation, but was oxidised in acetone solution with potassium permanganate in order, as far as possible, to remove all unsaturated esters. Consequently, as in the case of a rat body fat [Banks *et al.*, 1933], the mean equivalent and unsaturation of the unsaturated esters could then be calculated, after the proportion and mean equivalent of the saturated esters (which remain unaltered during the oxidation) had been determined.

When the hydrogenated portion of the "liquid" esters was fractionally distilled, it was found in all cases to contain comparatively large proportions of methyl palmitate and, indeed, much larger amounts of this ester than were isolated by the oxidation procedure to which reference has just been made. This fact, in addition to giving an alternative quantitative estimate for palmitoleic acid present (see below), affords definite proof of the existence in the original "liquid" esters of an unsaturated derivative of the normal C_{16} series of acids.

The experimental data from which the final compositions of the mixed fatty acids were calculated, by means of the two latter alternatives (oxidation of the primary "liquid" ester fraction or fractionation of the hydrogenated "liquid" esters), are illustrated in Table II. This gives the results obtained with the abdominal and gizzard fats from the group of 24 hens which had been reared on a fish meal-free diet.

From the proportions and equivalents of the saturated esters obtained by permanganate-acetone oxidation of the fractions L 1, the weights of the various esters present therein were estimated to be as follows:

Estimated composition of methyl ester fractions L 1.

	Abdominal layer fat (g.)	Gizzard fat (g.)
Myristate	0·19	Trace
Palmitate	2·37	3·14
Palmitoleate	7·18	10·02
Oleate	11·58	11·57
Linoleate	8·21	9·19

Apart from this, the calculation of the components of the various ester fractions followed the customary procedure, and it may suffice to record here the percentage compositions found for the respective "solid," "liquid" and "hydrogenated liquid" groups of fatty acids (Table III).

Table IV gives the corresponding data for the total component acids of the two fats, derived from a combination of the values found for the "solid" acids and for the "liquid" acids by direct ester fractionation (and determination of saturated esters in the lowest-boiling "liquid" ester fraction).

Table III. *Estimated percentage compositions of the "solid," "liquid" and "hydrogenated liquid" acids.*

	Abdominal layer fat % (wt.)	Gizzard fat % (wt.)
"Solid" acids S:		
Myristic	0·3	0·2
Palmitic	73·7	73·1
Stearic	22·0	22·7
Oleic	4·0	4·0
Unsaponifiable	Trace	Trace
"Liquid" acids L:		
Myristic	0·2	Trace
Palmitic	3·2	3·6
Palmitoleic	7·7	11·0
Oleic	55·9	51·6
Linoleic	31·9	33·1
C_{20-22} unsaturated	0·8	0·4
Unsaponifiable	0·3	0·3
"Hydrogenated liquid" acids HL:		
C_{16}	13·4	14·6
C_{18}	85·6	84·2
C_{20-22}	0·9	0·8
Unsaponifiable	0·1	0·4

Table IV. *Summarised data for component fatty acids of original abdominal and gizzard fats.*

Acid	Solid acids S	Liquid acids L	Total	Fatty acids (excluding unsaponifiable matter) % (wt.)	% (mol.)
		Abdominal layer fat.			
	(31·8 %)	(68·2 %)			
Myristic	0·11	0·14	0·25	0·3	0·3
Palmitic	23·42	2·15	25·57	25·6	27·3
Stearic	7·00	—	7·00	7·0	6·7
Palmitoleic	—	5·27	5·27	5·3	5·7
Oleic	1·27	38·13	39·40	39·4	38·2
Linoleic	—	21·77	21·77	21·8	21·3
C_{20-22} unsaturated	—	0·57	0·57	0·6	0·5
Unsaponifiable	—	0·17	0·17	—	—
		Gizzard fat.			
	(31·1 %)	(68·9 %)			
Myristic	0·05	Trace	0·05	0·1	0·1
Palmitic	22·73	2·46	25·19	25·2	26·9
Stearic	7·06	—	7·06	7·1	6·8
Palmitoleic	—	7·58	7·58	7·6	8·1
Oleic	1·25	35·55	36·80	36·9	35·6
Linoleic	—	22·81	22·81	22·8	22·3
C_{20-22} unsaturated	—	0·28	0·28	0·3	0·2
Unsaponifiable	0·01	0·22	0·23	—	—

Table V gives the corresponding figures for the mixed fatty acids, when the increments of acids in the "solid" group are combined with those for the "hydrogenated liquid" acids. In this case allowance has been made for the slight alteration in the proportions of the two groups, due to the proportionate increase in weight of the "liquid" acids as a result of hydrogenation.

Since hydrogenation of an unsaturated acid gives rise to the same number of molecules of the corresponding saturated acid, and since nearly all the

Table V. *Summarised data for combination of "solid" acids with "hydrogenated liquid" acids of abdominal and gizzard fats.*

Acid	Solid acids S	Liquid acids L	Total	Fatty acids (excluding unsaponifiable matter) % (wt.)	% (mol.)
		Abdominal layer fat.			
	(31·7 %)	(68·3 %)			
Myristic	0·11	—	0·11	0·1	0·1
Palmitic	23·35	9·18	32·53	32·5	34·9
Stearic	6·98	58·45	65·43	65·5	63·3
Oleic	1·26	—	1·26	1·3	1·2
C_{20-22}	—	0·59	0·59	0·6	0·5
Unsaponifiable	—	0·08	0·08	—	—
		Gizzard fat.			
	(30·9 %)	(69·1 %)			
Myristic	0·05	—	0·05	0·1	0·1
Palmitic	22·59	10·06	32·65	32·7	35·1
Stearic	7·01	56·36	63·37	63·6	61·4
Oleic	1·24	1·86	3·10	3·1	3·0
C_{20-22}	—	0·54	0·54	0·5	0·4
Unsaponifiable	0·01	0·28	0·29	—	—

palmitic acid in the original fats appears in the "solid" group which is common to both series of analyses, a practically independent value for the palmitoleic acid present can be derived by combining the molar percentages of palmitic acid in Table V with those of Table IV. The stearic acid present in the original fat is also entirely segregated in the "solid" group and (with the minor proportions of myristic and C_{20-22} acids observed in the results given in Table V) the acids remaining over must originally have been C_{18} unsaturated acids. The molar percentage of these is therefore now determined by difference (the relative proportions of oleic and linoleic acids being calculated on the basis of those observed in the C_{18} unsaturated esters actually obtained in the course of the analysis by the direct fractionation of the "liquid" esters). The corresponding deduced molar (and thence weight) percentages of the acids present in the original fats, thus derived from the results of the analysis of the "hydrogenated liquid" esters, are given in Table VI, and it will be seen that the palmitoleic acid figures in this table and those in Table IV are in good agreement.

Table VI. *Alternative calculation of component acids from the palmitic acid figures before (Table IV) and after (Table V) hydrogenation.*

Acid	Abdominal layer fat % (mol.)	whence % (wt.)	Gizzard fat % (mol.)	whence % (wt.)
Myristic	0·1	0·1	0·1	0·1
Palmitic	27·3	25·6	26·9	25·3
Stearic	6·7	7·0	6·8	7·1
Palmitoleic	7·6	7·0	8·2	7·6
Oleic	37·1	38·4	35·4	36·6
Linoleic	20·7	21·3	22·2	22·8
C_{20-22} unsaturated	0·5	0·6	0·4	0·5

We have examined several of the individual ester fractions obtained in order to satisfy ourselves as to the identity of individual acids present.

(*a*) The fatty acids from the residual ester fractions L 7, after removal of unsaponifiable matter, were brominated in dry ethereal solution, when a very small amount of an insoluble bromo-addition product was precipitated, which was insoluble in boiling benzene and which decomposed without melting at about 220–230°. These properties are characteristic of the polybromo-addition products of highly unsaturated acids of the C_{20} or C_{22} series.

(*b*) Two fractions (L 4 and L 5) of "liquid" esters which appeared to consist wholly of C_{18} unsaturated esters were examined, after hydrolysis, with reference to the unsaturated acids present:

The acids (2·14 g.) from L 4 yielded no bromo-addition products insoluble in ether, but gave 0·42 g. of a substance, sparingly soluble in light petroleum, which, after crystallisation from the latter solvent, melted at 114° and showed no depression in melting-point when mixed with the tetrabromostearic acid isolated in the same manner from the mixed acids of cottonseed oil.

The potassium salts of the acids from L 5 were oxidised with dilute ice-cold alkaline permanganate according to Lapworth and Mottram [1925, 1]. From the products of oxidation there were isolated: (i) a tetrahydroxystearic acid (Found: C, 62·0; H, 10·0 %. $C_{18}H_{36}O_6$ requires: C, 62·1; H, 10·3 %) sparingly soluble in hot, and insoluble in cold, water, M.P. 168–171° (unchanged on admixture with the tetrahydroxystearic acid, M.P. 173°, obtained from the linoleic acid of cottonseed oil); and (ii) the dihydroxystearic acid, M.P. 130–131° (unchanged when mixed with an authentic specimen from ordinary oleic acid).

(*c*) As already stated, the saturated acids present in the lowest-boiling fractions resulting from redistillation of the fractions L 1 were exhaustively examined by fractional crystallisation in several cases. Several crops of crystals were obtained, the least soluble of which melted at 60·5–62·0° (unchanged when mixed with palmitic acid), whilst the most soluble acids melted at 46–47°; since this melting-point was raised to 52·5–55·5° by admixture with palmitic acid and to 47·5–50·0° by admixture with myristic acid, whilst addition of lauric acid depressed it to below 35°, it is clear that the only saturated components present were the two former acids.

(*d*) Identification of the saturated C_{16} acid produced during hydrogenation of the "liquid" esters was also attempted, but the lowest-boiling ester fractions (HL 1, equivalent 279–280) already contained much stearate, with the result that complete isolation of palmitic acid could not be effected. However, crystallisation from alcohol of the mixed acids (2·77 g.) from an ester fraction HL 1 gave a least soluble crop of crystals (1·55 g., M.P. 61–63°) and a second crop (0·73 g.) which melted sharply at 55°. The latter crop, the melting-point of which was raised on admixture with an equal weight of either palmitic or stearic acid, was evidently the characteristic palmitic-stearic acid "eutectic" mixture, whilst the first crop was similarly shown by mixed melting-point tests to be a mixture of palmitic with an excess of stearic acid. No indication was obtained of the presence of any acids other than palmitic and stearic.

(*e*) Finally, after the quantitative analyses had been completed, a mixture of the abdominal (500 g.) and gizzard (100 g.) fats from the hens fed on a fish meal-free diet was hydrolysed and submitted to the lead salt separation. The "liquid" acids were converted into methyl esters, and the lowest-boiling fractions were refractionated several times in order to obtain a mixture of esters as free as possible from C_{18} unsaturated components.

The specimen finally obtained (14·8 g., sap. equiv. 270·5, I.V. 80·3) was submitted to oxidation (and re-oxidation) in acetone solution with potassium permanganate. Unchanged saturated esters (4·02 g., sap. equiv. 264·2, I.V. 2·8)

were recovered, which indicated that the ester fraction oxidised was composed of saturated (4·0 g.), palmitoleic (8·7 g.) and C_{18} unsaturated (2·1 g.) esters.

The acidic products from the two oxidations were united, completely hydrolysed, and the mixture of mono- and di-basic acids (9·1 g.) was separated by cooling their light petroleum solution at 0°. The insoluble crystalline dibasic acids (6·2 g.) were crystallised from water and finally from ethyl acetate, when the product (3·1 g.) melted at 95–98°[1] (unchanged on admixture with azelaic acid) and had an equivalent of 93·2 (azelaic acid requires 94·0). The maximum possible yield of azelaic acid from the 2·1 g. of C_{18} unsaturated esters present in the substance oxidised is about 1·4 g., so that azelaic acid must have been produced in quantity from the palmitoleic ester. Moreover, the material (2·9 g.) which remained in solution in light petroleum was recovered and distilled at atmospheric pressure, when a distillate (1·4 g.), which came over at 220–225°, and possessed an equivalent of 133·0, was obtained (*n*-heptanoic acid, mol. wt. 130, boils at 223°). The residue from this distillation was solid at the ordinary temperature and was probably mainly azelaic acid, which is not completely insoluble in ice-cold light petroleum.

The palmitoleic acid of the hen fats is therefore the same as that which has been identified in various marine animal oils, and possesses the constitution $CH_3.[CH_2]_5.CH:CH.[CH_2]_7.COOH$.

It has been necessary to go into the foregoing details in consequence of the somewhat unusual character of the mixed fatty acids of the bird fats; but we may now proceed to tabulate the final results obtained for the abdominal, gizzard and neck fats of the two groups of birds. Table VII shows the data for

Table VII. *Component fatty acids of depot fats from group of 24 Light Sussex hens (age 7 months).*

Acid	Weight percentages			Molar percentages		
	Abdominal	Gizzard	Neck	Abdominal	Gizzard	Neck
(i) Calculated from the direct analysis.						
Myristic	0·3	0·1	0·3	0·3	0·1	0·3
Palmitic	25·6	25·2	26·7	27·3	26·9	28·4
Stearic	7·0	7·1	5·9	6·7	6·8	5·7
Palmitoleic	5·3	7·6	6·6	5·7	8·1	7·1
Oleic	39·4	36·9	39·0	38·2	35·6	37·6
Linoleic	21·8	22·8	21·2	21·3	22·3	20·6
C_{20-22} unsaturated	0·6	0·3	0·3	0·5	0·2	0·3
(ii) Calculated from the results of analysis of the "hydrogenated liquid" esters.						
Myristic	0·1	0·1	—	0·1	0·1	—
Palmitic	25·6	25·3	—	27·3	26·9	—
Stearic	7·0	7·1	—	6·7	6·8	—
Palmitoleic	7·0	7·6	—	7·6	8·2	—
Oleic	38·4	36·6	—	37·1	35·4	—
Linoleic	21·3	22·8	—	20·7	22·2	—
C_{20-22} unsaturated	0·6	0·5	—	0·5	0·4	—

the fats from the group of hens whose diet included no fish meal, whilst Table VIII shows the corresponding figures for the fats from the 12 hens which received 7 % of fish meal in their diet.

[1] A small amount of suberic acid (M.P. 132–136°, mixed M.P. 134–136°) was present with the azelaic acid; this was probably formed by further oxidation of a little dihydroxypalmitic acid produced during the initial oxidation [*cf.* Lapworth and Mottram, 1925, 2].

Table VIII. *Component fatty acids of depot fats from group of 12 Light Sussex hens (age 2 years).*

Acid	Weight percentages			Molar percentages		
	Abdominal	Gizzard	Neck	Abdominal	Gizzard	Neck
(i) Calculated from the direct analysis.						
Myristic	1·2	0·6	1·2	1·5	0·8	1·4
Palmitic	24·0	25·4	24·5	25·5	27·0	26·1
Stearic	4·1	4·2	4·2	3·9	4·0	4·0
Palmitoleic	6·7	7·1	6·9	7·2	7·7	7·4
Oleic	42·5	43·0	42·8	41·0	41·5	41·2
Linoleic	20·8	18·4	20·4	20·3	17·9	19·9
C_{20-22} unsaturated	0·7	1·3	Trace	0·6	1·1	Trace
(ii) Calculated from the results of analysis of the "hydrogenated liquid" esters.						
Myristic	0·9	0·3	—	1·1	0·4	—
Palmitic	24·0	25·4	—	25·5	27·0	—
Stearic	4·1	4·2	—	3·9	4·0	—
Palmitoleic	8·5	8·0	—	9·1	8·6	—
Oleic	41·3	43·2	—	39·9	41·7	—
Linoleic	20·3	18·5	—	19·7	18·0	—
C_{20-22} unsaturated	0·9	0·4	—	0·8	0·3	—

SUMMARY AND DISCUSSION.

The present work has shown that hen body fats possess specific characteristics which distinguish them from those of quadrupeds and also from those of fishes or marine mammals. They fall into one of the two broad groups of depot fats which are found in non-aquatic animals [*cf.* Banks *et al.*, 1933], namely, the class in which the component fatty acids contain only about 30–35 % of saturated acids. They are definitely not of the more saturated type found in the pig, ox, sheep, horse, *etc.*, in which the proportion of stearic acid is variable but usually considerable. In the hen depot fats, about 65 % of the component acids belong to the C_{18} series, but these are mainly oleic (35–38 %) and linoleic (20–22 %) acids; no evidence of acids more unsaturated than linoleic has been observed in the C_{18} group.

The presence of unsaturated C_{20-22} acids (although in very small amounts), but still more the occurrence of notable proportions (7–8 %) of palmitoleic acid, causes these bird depot fats to possess features which are somewhat reminiscent of those of marine animal fats. Indeed, the component acids of the hen fats may be regarded as intermediate in character between those of the fats of land and marine animals, but they are much closer to such fats as those of the rodents than to those of aquatic fauna.

It may be pointed out that palmitoleic acid (apart from its presence in the fats of aquatic animals) has only been reported up to the present in hen and rat body fats and in the fats of diphtheria bacilli [Chargaff, 1933] and of lycopodium spores [Riebsomer and Johnson, 1933].

The presence of 65–68 % of unsaturated acids in the component acids of the depot fats of an animal with so high a body temperature as the hen affords another interesting example of the fact that body temperature is by no means the sole determining factor of the relative saturation of depot fats. At the same time, the almost identical compositions of the fats from three different parts of the body of the birds, one of these being situated much farther from the outer skin than the others, may be contrasted with the progressive change in relative saturation on passing, for example, from the perinephric fat of the pig to the fat

lying nearest to the skin; and here it may well be that the more uniform body temperature of the feathered animal leads to the absence of any regional difference in depot fat composition. The influence of the body temperature is thus to be regarded as a secondary factor which may modify the composition of depot fat; but the specific type of the latter seems to depend primarily on the biological species.

Amongst the saturated acids of the hen fats, myristic acid is present in little more than traces, and stearic acid forms only 5–7 % of the total component acids. It is very interesting to observe however that, despite the specific features which seem to distinguish hen depot fats, their palmitic acid content is exactly of the same order (25–30 mol. %) as that found in practically all land animal fats, whether relatively saturated or unsaturated, studied up to the present. It is perhaps well to emphasise once more the regularity with which palmitic acid falls within these limits in the depot fats of land animals of widely varying types, and also to point out that a palmitic acid content of 25–30 % is practically never met with in the fats of marine animals and is only approached in very few instances in vegetable seed fats (in fruit coat fats the presence of even higher proportions of palmitic acid is of course less uncommon [Hilditch, 1933]).

Finally, we have shown that the occurrence of 7–8 % of palmitoleic acid in the hen fats is not dependent on the diet received by the birds. Within the limits of the experimental analytical error, all the six depot fats are closely similar. The only general differences between the two groups are that the proportions of stearic and linoleic acids are possibly slightly lower, and those of myristic, oleic and the C_{20-22} acids perhaps somewhat higher, on the whole, in the fats from the older birds (whose diet included fish meal) than in those of the others. This observation is indeed probably of greater interest in relation to the marked difference in age of the two groups of birds (7 months and 2 years respectively) than to any slight difference in their diets. The characters of the depot fats are the same both in young and in mature birds, fed on similar rations.

All the foregoing observations of course refer to birds which have received a diet of which fat formed only a minor proportion (4 % or less).

Our thanks are offered to the Department of Scientific and Industrial Research for a grant in aid of this investigation.

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