The Structure of the Naturally Occurring Phosphoglycerides

1. EVIDENCE DERIVED FROM ALKALINE-HYDROLYSIS STUDIES

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The structures hitherto assigned to the lecithins and other naturally occurring phosphoglycerides have been largely based on the analysis of acid or alkaline hydrolysates. The presence in these hydrolysates of a mixture of α - and β -glycerophosphates was formerly taken as evidence for the occurrence of both an α - and β -series of phosphoglycerides in nature (Karrer & Salomon, 1926; Suzuki & Yokoyama, 1930; MacLachlan *et al.* 1942; Welch, 1945; Burmaster, 1946c).

Bailly (1939), however, had earlier found that interconversion of α - and β -glycerophosphates takes place when either substance is heated in acid solution, and Folch (1942a) noted that several phosphoglycerides from different sources always gave the same ratio of α - and β -glycerophosphate concentrations when subjected to acid hydrolysis under standard conditions. Burmaster (1946c), employing a fractionation procedure stated by Suzuki & Yokoyama (1930) to effect a separation of naturally occurring phosphoglycerides into an α - and β -series, found that on alkaline hydrolysis each of the supposedly pure α - and β -fractions yielded a mixture of glycerophosphates which always contained the same ratio of α - to β -isomers. More recently, Baer & Kates (1948) have demonstrated that both the acid and alkaline hydrolysis of synthetic L- α -glycerylphosphorylcholine is accompanied by migration of the phosphate group, resulting in the formation of a mixture of β -, D- α - and L- α -glycerophosphates. Finally, Baer & Kates (1950a) have shown that the acid or alkaline hydrolysis of a series of synthetic L- α -lecithins is accompanied by a similar phosphate migration. That a migration of the phosphate group likewise occurs when naturally occurring lecithins are hydrolysed by acid or alkali seems certain, thus accounting for the formation of both the α - and β -glycerophosphates reported in the literature. It is interesting to note that an analogous case of phosphate migration has been encountered in the formation of isomeric mononucleotides by chemical hydrolysis of the naturally occurring ribonucleic acids (Cohn, 1950a, b; Brown, Haynes & Todd, 1950; Brown & Todd, 1952a, b).

In the work described in this paper, we have determined the proportions of β -, D- α - and L- α -

glycerophosphates formed on acid and alkaline hydrolysis of three partially purified naturally occurring lecithins and of a sample of ox-brain 'kephalin', and have compared them with the hydrolysis products of synthetic phosphoglycerides of known constitution. We are not aware of any previously published complete analyses of the glycerophosphates obtained on chemical hydrolysis of naturally occurring phosphoglycerides. The results show clearly that on alkaline hydrolysis the natural phosphoglycerides behave in the same way as synthetic L- α -lecithin, but quite differently from synthetic DL- α -lecithin, DL- α -kephalin or β -kephalin. A preliminary account of these results has already appeared (Maguire, 1952).

Further work is now in progress in which natural lecithins are being subjected to enzymic hydrolysis, and the results of these experiments will be reported in subsequent papers.

EXPERIMENTAL

Analytical methods

Total N was determined in the distillation apparatus of Markham (1942) after micro-Kjeldahl digestion, using the catalyst of Chibnall, Rees & Williams (1943). Choline was estimated as the reineckate on Ba(OH)₂ hydrolysates by the procedure of Glick (1944). Ethanolamine N + serine N was determined on acid hydrolysates by alkaline periodate oxidation in Conway microdiffusion units and measurement of the NH₃ liberated (Burmaster, 1946b). Total P was determined as orthophosphate, after ashing about 100 μ g. phosphoglyceride with 0.4 ml. 72% HClO₄, by the method of Berenblum & Chain (1938), using the micromodification described by Long (1943). Ammonium dihydrogen phosphate of A.R. purity was used as standard for both N and P determinations.

Materials

Phosphoglycerides from natural sources. A sample of ox-heart lecithin and several specimens of ovolecithin were isolated by the method of Pangborn (1951).

Soyabean lecithin was prepared from an acetone-insoluble fraction of soyabean lipids (Bibby and Co. Ltd., Liverpool), using the following procedure. The material (25 g.) was shaken with ethanol (500 ml.) at room temperature for 6 hr. After filtering off insoluble material, the extract was treated with 50% (w/v) CdCl₂ solution until precipitation was complete. Subsequent purification was carried out according to Pangborn (1951). Yield, 0.6 g.

Ox-brain 'kephalin' was prepared by the method of Folch (1942*b*). After removing the 'inositol phosphatide', the product obtained corresponded with 'fractions III-V', i.e. a mixture in which phosphatidylethanolamine and phosphatidylserine predominate.

All lecithin samples were dissolved in ethanol; the ox-brain 'kephalin' was dissolved in CHCl_3 . These solutions (approx. 30 mg. dry wt./ml.) were stored in glass-stoppered flasks at 0°.

Analytical figures relating to these natural phosphoglycerides are given in Table 1. In spite of the unsatisfactory analysis shown by the soyabean lecithin, studies with this substance have been included since it behaved like the other lecithins on acid and alkaline hydrolysis.

Table 1. *Analysis of natural phosphoglycerides*

	Total N (%)	Choline N (%)	Hydroxy- amino N (%)	Total P (%)
Ovolecithin	1.86	1.72	0.04	3.94
Ox-heart lecithin	1.89	1.72	0.06	3.93
Soyabean lecithin	0.80	0.27	—	4.25
Ox-brain kephalin	1.53	0.12	0.99	3.41

Synthetic phosphoglycerides. (For consistency of nomenclature, the designations α and β refer to the position of attachment of the phosphate group to the glycerol molecule.) The following samples of synthetic phosphoglycerides were used: dipalmitoyl DL- α -lecithin, dimyristoyl DL- α -kephalin, dimyristoyl β -kephalin (Malkin & Bevan, 1951) and dimyristoyl L- α -lecithin (Baer & Kates, 1950*b*). The term 'kephalin' is generally applied to a mixture of naturally occurring substances, containing both phosphatidylethanolamine and phosphatidylserine, but the synthetic kephalins used in this work were the ethanolamine derivatives only.

Barium α -glycerophosphates. Barium L- α -glycerophosphate (L- α -GPA) (Baer & Fischer, 1939) and barium DL- α -glycerophosphate (DL- α -GPA) (King & Pyman, 1914) were used as standards.

L- α -Glycerophosphate dehydrogenase. An active enzyme, obtained from fresh sheep brain by the method of Weil-Malherbe (1937), was dispersed in 0.1M-sodium veronal acetate buffer, pH 8.0 (Michaelis, 1931), and stored at 0°. There was no loss of activity during 3–4 weeks.

Hydrolysis of phosphoglycerides and isolation of mixed glycerophosphates

Into a series of Pyrex tubes (19 × 150 mm.), containing a solution of the phosphoglyceride (80–90 mg. dry wt.), were pipetted 2 ml. of either N-NaOH, saturated aqueous $\text{Ba}(\text{OH})_2$ or N-HCl. Lecithin samples were always first dissolved in 0.5 ml. ethanol, while the 'kephalins' were dissolved in 0.5 ml. CHCl_3 . Closely fitting cold fingers were used as condensers. The tubes were then placed in an oil bath at 110–120°. Heating was continued for 2, 3 and 7 hr. using NaOH, $\text{Ba}(\text{OH})_2$ and HCl hydrolysis, respectively. This is essentially the procedure of Baer & Kates (1950*a*) on a reduced scale.

At the end of the appropriate time, solvent was removed by withdrawing the cold finger. Each hydrolysate was then treated with distilled water (5 ml.) and neutralized with HCl or NaOH. Free fatty acids were precipitated by addition of 1 ml. 15% (w/v) trichloroacetic acid (TCA); after standing

at room temperature for 15 min., they were filtered off and the filtrate collected in a 60 ml. centrifuge tube. The precipitate was washed twice with 2 ml. portions of 2.5% TCA. The combined filtrate and washings were treated with 2N-barium acetate (0.5 ml.) and brought to pH 8.4 (phenolphthalein) with 10N-NaOH. Ethanol (3 vol.) was then added and after thorough mixing, the stoppered tube was chilled overnight. The precipitate of barium glycerophosphates was spun down and the supernatant discarded. The precipitate was redissolved in 3 ml. water with the addition of the minimum amount of HCl. The solution was then transferred to a 15 ml. graduated centrifuge tube, the pH adjusted to 8.4 and the barium glycerophosphate reprecipitated with 3 vol. ethanol. After chilling for 2–3 hr. the precipitate was spun down and dried *in vacuo* over H_2SO_4 . The dry solid was then dissolved in 1 ml. 0.01N-HCl and the solution stored at 0° until required for analysis. The barium glycerophosphates isolated in this way contained not less than about 85% of the original phosphoglyceride P.

Determination of total glycerophosphate

Total glycerophosphate (β , D- α and L- α) was determined by a micromodification of the method of Burmaster (1946*a*). A sample of the barium glycerophosphate solution (equivalent to about 3.5 μg . P) was treated with 1.4M-HClO₄ (1 ml.) followed by 0.01M-KIO₄ (0.2 ml.), and the mixture placed in a boiling-water bath for 1 hr. A water blank and inorganic phosphate standard were similarly treated. Inorganic phosphate estimations were carried out on the cooled solutions. The method gave 100% of the theoretical values, based on total P content, when tested with the two samples of synthetic barium α -glycerophosphate and with a commercial preparation of sodium β -glycerophosphate.

Determination of α -glycerophosphate

The amount of total α -glycerophosphate (D- α and L- α) was determined by a periodate oxidation procedure based on the method of Fleury & Lange (1933), in which α -glycerophosphate is rapidly and quantitatively oxidized by one equivalent of HIO₄ at room temperature, while β -glycerophosphate remains unattacked.

The barium glycerophosphate solution (0.1 ml., equivalent to 100–250 μg . α -glycerophosphate P), was treated with 1 ml. approx. 0.02N-KIO₄ and the mixture, adjusted to pH 4, allowed to stand for 1 hr. To the solution was then added 2 drops of M-Na₂SO₄ (to precipitate Ba) followed by 2 drops of M-NaHCO₃ to raise the pH into the range suitable for the selective titration of the excess KIO₄ in the presence of KIO₃. Solid KI (about 0.1 g.) was then added and the liberated iodine titrated with a solution of standard 0.02N-sodium arsenite in 0.1M-phosphate buffer, pH 7.0 (Washburn, 1908), using 2 drops 1% starch solution as indicator. A blank titration on 1 ml. of the KIO₄ solution was carried out at the same time, the difference between this and the experimental value being used to calculate the α -glycerophosphate content of the solution. This method gave theoretical results with the samples of synthetic barium L- α - and DL- α -glycerophosphates.

Determination of L- α -glycerophosphate

The amount of L- α -glycerophosphate contained in a phosphoglyceride hydrolysate was determined manometrically by measuring the O₂ uptake in the presence of

L- α -glycerophosphate dehydrogenase with pyocyanine (Weil-Malherbe, 1937) as O_2 carrier. Conventional Warburg technique was employed.

The main chamber of the Warburg vessel contained 2.0 ml. buffered enzyme dispersion, pH 8.0, and 0.5 ml. 0.02M-pyocyanine, a filter-paper roll soaked in 2N-KOH (0.2 ml.) being placed in the centre well. Into the side bulb was pipetted 0.2–0.4 ml. barium glycerophosphate solution (equivalent to approx. 150 μ g. of L- α -glycerophosphate P) and 0.1 ml. 0.01M-phenol red. This mixture was then titrated with 0.1N-NaOH until the indicator turned a definite pink colour (pH about 8.0); distilled water was added to bring the total fluid volume in the side bulb to 0.7 ml. After 15 min. temperature equilibration at 38°, the contents of the side bulb were tipped into the main chamber and readings taken until no further O_2 uptake occurred; the reaction was usually completed within 45 min. The final reading was corrected for the small blank O_2 uptake of the enzyme without added substrate.

Theoretical O_2 uptakes were obtained with known amounts of DL- α - and L- α -glycerophosphates.

RESULTS

Acid hydrolysis

When an acidified solution of sodium β -glycerophosphate is heated to boiling for 1 hr., migration of the phosphate group occurs and equilibrium is reached when about 85 % has been converted into DL- α -glycerophosphate (Bailly, 1939). It follows, therefore, that acid hydrolysis of all phosphoglycerides, whether they be the synthetic β -, L- α - or DL- α -compounds or the naturally occurring substances will also give an equilibrium mixture of about 15 % β -glycerophosphate and 85 % DL- α -glycerophosphate. Therefore, analysis of the products of acid hydrolysis can give no information regarding the configuration of the phosphoglycerides. In spite of this fact, we have found it valuable to subject all our samples of naturally occurring phosphoglycerides to acid hydrolysis as a routine, for any deviation from the expected relative proportions of the glycerophosphates would indicate either the presence of some interfering impurity in the materials used or a failure of the analytical procedure. Thus, when a sample of crude ox-brain 'kephalin', prepared by the method of Folch (1942*b*), was hydrolysed in N-HCl under the conditions already defined, the results obtained were not those to be expected for a simple phosphoglyceride. The discrepancy was found to be due to the presence of 'inositol phosphatide', which on acid hydrolysis yields a water-soluble inositol phosphate, forming an alcohol-precipitable barium salt, which in turn reacts with potassium periodate at room temperature. The net result was an erroneously high value for the apparent α -glycerophosphate; indeed, the observed value was appreciably higher than that found for total glycerophosphate. When the 'inositol phosphatide' was removed by

ethanol precipitation, however, to give Folch's 'fractions III–V', normal values were found after acid hydrolysis. All the naturally occurring phosphoglycerides used in the present work were ethanol-soluble, so that there was no possibility of interference from contaminating 'inositol phosphatide'. Results of the acid-hydrolysis studies are shown in Table 2. It will be noted that the α -glycerophosphate is always present in the racemic form.

Table 2. *Composition of glycerophosphates formed on acid hydrolysis of synthetic and naturally occurring phosphoglycerides*

(Acid hydrolysis carried out by refluxing with N-HCl at 110–120° for 7 hr. Determination of total, α - and L- α -glycerophosphates, as given in Experimental section. Values are given as percentage of total glycerophosphate.)

	Analysis of glycerophosphates			
	β	α	L- α	D- α
(a) Synthetic phosphoglycerides				
Dimyristoyl L- α -lecithin	10	90	46	44
Dipalmitoyl DL- α -lecithin	14	86	44	42
Dimyristoyl DL- α -kephalin	12	88	43	45
Dimyristoyl β -kephalin	12	88	43	45
(b) Naturally occurring phosphoglycerides				
Ovolecithin	11	89	46	43
Ox-heart lecithin	13	87	44	43
Soyabean lecithin	18	82	39	43
Ox-brain kephalin	15	85	42	43

Alkaline hydrolysis

The phosphate group does not migrate when α - or β -glycerophosphates are heated in alkaline solution (Verkade, Stoppelenburg & Cohen, 1940). Partial isomerization does occur, however, when synthetic L- α -glycerylphosphorylcholine (Baer & Kates, 1948) or L- α -lecithins (Baer & Kates, 1950*a*) are subjected to alkaline hydrolysis. The α -glycerophosphate is, however, almost entirely in the L form. It has been found in the present work that partial isomerization also takes place when synthetic DL- α -lecithin, DL- α -kephalin and β -kephalin are hydrolysed with alkali, and that the composition of the glycerophosphate mixture differs from that formed from L- α -lecithin in that the α -glycerophosphate is racemic. The naturally occurring phosphoglycerides on alkaline hydrolysis all yield glycerophosphate mixtures similar to that found with L- α -lecithin and differing from that found with β - and DL- α -phosphoglycerides. These results are summarized in Table 3.

DISCUSSION

The only experimental procedure used in this work which requires comment is the use of the L- α -glycerophosphate dehydrogenase for the specific

Table 3. *Composition of glycerophosphates formed on alkaline hydrolysis of synthetic and naturally occurring phosphoglycerides*

(Alkaline hydrolysis carried out by refluxing with N -NaOH for 2 hr. or sat. $Ba(OH)_2$ for 3 hr., at 110–120°. Determination of total, α - and L - α -glycerophosphates, as given in Experimental section. Values are given as percentage of total glycerophosphate.)

	Analysis of glycerophosphates after hydrolysis with							
	NaOH				$Ba(OH)_2$			
	β	α	L - α	D - α	β	α	L - α	D - α
(a) Synthetic phosphoglycerides								
Dimyristoyl L - α -lecithin	51	49	43	6	56	44	39	5
Dipalmitoyl DL - α -lecithin	53	47	23	24	52	48	24	24
Dimyristoyl DL - α -kephalin	50	50	26	24	50	50	26	24
Dimyristoyl β -kephalin	52	48	22	26	55	45	23	22
(b) Naturally occurring phosphoglycerides								
Ovolecithin	53	47	41	6	54	46	42	4
Ox-heart lecithin	52	48	42	6	54	46	40	6
Soyabean lecithin	53	47	42	5	54	46	42	4
Ox-brain kephalin	51	49	47	2	53	47	42	5

determination of L - α -glycerophosphate, present in the phosphoglyceride hydrolysates. This method was necessarily adopted because polarimetric analysis, used successfully by Baer & Kates (1950a) on hydrolysed samples of synthetic lecithins, appeared unsuitable for the determination of small amounts of L - α -glycerophosphoric acid of specific optical rotation -1.2° , where the presence of traces of optically active impurities would render the method useless. The validity of the enzymic method has been confirmed by the fact that theoretical values were obtained with pure DL - α - and L - α -glycerophosphates and moreover the values obtained in this way on hydrolysates of a synthetic L - α -lecithin were identical with those published by Baer & Kates (1950a) using their polarimetric method.

The main result which emerges from this study is that on alkaline hydrolysis the naturally occurring phosphoglycerides and synthetic dimyristoyl L - α -lecithin give exactly the same proportions, of β -, L - α - and D - α -glycerophosphates. Since, on the other hand, dipalmitoyl DL - α -lecithin, dimyristoyl DL - α -kephalin and dimyristoyl β -kephalin give different proportions of the L - α - and D - α -glycerophosphates, it seems reasonable to infer that the naturally occurring phosphoglycerides belong to the L - α -series. This conclusion is consistent with the observation that the so-called 'hydrolecithin', i.e. dipalmitoyl lecithin, first isolated from the larvae of the tapeworm *Cysticercus fasciolaris* (Lesuk & Anderson, 1941) and later from several ox tissues (Thannhauser, Benotti & Boncoddio, 1946; Thannhauser & Boncoddio, 1948) appears to be identical with synthetic dipalmitoyl L - α -lecithin (Baer & Kates, 1950b). Furthermore, the glycerylphosphorylcholine, isolated by Schmidt, Hershman & Thannhauser (1945) from autolysed pancreas and assumed to have arisen by the action of lecithinases A and B

on the endogenous lecithin, was likewise stated to possess the L - α -configuration. It is also interesting to note that the naturally occurring 'acetal phosphatide' of ox brain, on catalytic removal of the fatty aldehyde group with aqueous mercuric chloride, gives only L - α -glycerylphosphorylethanolamine, showing that this phosphoglyceride also belongs to the L - α -series (Thannhauser, Boncoddio & Schmidt, 1951).

Finally, it seems desirable to consider briefly the mechanism by which the various isomeric glycerophosphates arise. Baer & Kates (1950a) have shown that when a synthetic L - α -lecithin is subjected to alkaline hydrolysis, the fatty acids are liberated much more rapidly than is the free choline, an observation which clearly indicates that the main intermediate product is L - α -glycerylphosphorylcholine (L - α -GPC). Presumably, synthetic β -phosphoglycerides will likewise yield β -glycerophosphorylcholine (β -GPC) as intermediate product.

Two theories have been proposed to explain the mechanism of alkaline hydrolysis of phosphate diesters, such as the GPC's, which contain an adjacent hydroxyl group. In the scheme of Baer & Kates (1950a), L - α -GPC is converted via the L -cyclic ortho triester into β -GPC, which in turn may give rise to both D - α - and L - α -GPC by way of the respective D - and L -cyclic ortho triesters. These three isomeric GPC's are then hydrolysed to the corresponding glycerophosphates. The theory does not imply, of course, that an equilibrium mixture of the three GPC's is formed before any choline is split off; the removal of choline may proceed simultaneously with the isomerization reactions. This theory, however, seems unable to account for the different ratios of β -GPA to D - α -GPA which have been found in the alkaline hydrolysates of synthetic β - and L - α -phosphoglycerides in the present work. Since it

is postulated that β -GPA and D- α -GPA can only arise through the intermediate formation of β -GPC, then the same ratio would be expected indepen-

glycerophosphates on alkaline hydrolysis, as has been found by experiment. This mechanism of hydrolysis can be represented as follows:

dently of whether the β -GPC originates from a β - or L- α -phosphoglyceride.

Brown & Todd (1952*b*) have suggested that loss of choline is more likely to take place from the cyclic ortho triesters than from the open chain GPC's. In the case of L- α -GPC hydrolysis, conversion to the L-cyclic ortho triester, loss of choline to give a cyclic diester and subsequent ring opening would yield a mixture of β -GPA and L- α -GPA. If it is assumed that the cyclic diesters have about equal chances of splitting at either of the two ester bonds, then approximately equal amounts of β -GPA and L- α -GPA will be formed. The conversion of some of the L-cyclic ortho triester by way of β -GPC to the D-cyclic ortho triester and degradation of the latter to β -GPA and D- α -GPA would readily account for the small quantity of D- α -GPA found in alkaline hydrolysates of L- α -GPC. In the alkaline hydrolysis of β -GPC, on the other hand, there will be formed 50% each of the L- and D-cyclic ortho triesters. Making the same assumption as before, the former will give rise to 25% each of β -GPA and L- α -GPA, while the latter will yield 25% each of β -GPA and D- α -GPA. It will be seen that these calculations for the breakdown of β -GPC and L- α -GPC are consistent with the results obtained for the alkaline hydrolysis of β - and L- α -phosphoglycerides, shown in Table 3. By the same reasoning it may be shown that β - and DL- α -phosphoglycerides should give the same mixture of

The experimental results obtained in the present work may therefore be regarded as providing support for the validity of the theory of Brown & Todd (1952*b*).

SUMMARY

1. The amount of L- α -glycerophosphate, in an acid or alkaline hydrolysate of a phosphoglyceride, has been determined manometrically, using the L- α -glycerophosphate dehydrogenase of sheep brain.

2. Alkaline hydrolysis, using either N-sodium hydroxide or saturated aqueous barium hydroxide, of four naturally occurring phosphoglycerides, yields proportions of β -, L- α - and D- α -glycerophosphates identical with those given by synthetic dimyristoyl L- α -lecithin and quite distinct from those obtained on alkaline hydrolysis of synthetic β - or DL- α -phosphoglycerides. This observation indicates that the naturally occurring phosphoglycerides belong to the L- α -series.

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Animal Fats

1. THE COMPONENT ACIDS OF DEER FAT AND OF CAMEL FAT

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Study of the detailed composition of seed fats has led to the realization that there is a close relationship between the classification of the parent plants on the basis of fat composition and that arrived at by botanists on more general grounds. Among land animals, however, only very tentative generalizations can be made because of the paucity of detailed analyses of animal fats. Hilditch (1947, p. 85) has drawn attention to this deficiency and it has been further emphasized by Barker & Hilditch (1950) who list the land animals, the fats of which have been satisfactorily analysed. Further analyses have since been reported for the fats obtained from hippopotamus (Barker & Hilditch, 1950), badger (Gupta, Hilditch & Meara, 1950), horse (Brooker & Shorland, 1950; Gupta & Hilditch, 1951), rabbit (Clément & Meara, 1951) and tiger (Pathak & Agarwal, 1952).

We are interested in this problem, and over the last few years have collected, from fifteen different animals, samples of fats which we hope to study. These vary in weight from 20 to 600 g. and cover an iodine-value range from 26 to 108. The fats from many of these animals have not previously been examined in any detail, and even in the few cases where satisfactory analyses have been reported it is considered worth while to repeat the work in order to study variation between different members of the same species. In the present paper we record our analyses of deer and of camel fat; these relatively simple examples were purposely selected as a convenient beginning to this work in order to gain experience for the study of more complicated fats. The methods of analysis may have to be varied

for different fats. Modifications have already been made in the light of experience and the methods reported in this paper are, therefore, not in every detail the most satisfactory ones. Improvements will be reported later as they are applied.

Reports on the composition of deer fat seem to be limited to the recording of physical data and characteristics (largely summarized by Lewkowitsch, 1922) and to the work of Baughman, Jamieson & McKinney (1929) and of Treadwell & Eckstein (1939). The former authors studied five samples of American reindeer fat of iodine value 33.7–39.4. Treadwell & Eckstein have reported the saponification value, iodine value and thiocyanogen value of fats obtained from various parts of five Virginia white-tailed deer (*Odocoileus virginianus borealis*). Apart from the fat isolated from the mammary gland there is little variation in these samples. These earlier results are summarized in Table 8.

Previous recorded data on camel fat appear to be limited to a report by Armstrong & Allan (1924), in which only the major component acids, palmitic (37%), stearic (16%) and oleic (47%) are recognized.

EXPERIMENTAL

Preliminary work

In planning this series of analyses, it was anticipated that some of the fatty-acid fractions to be examined would be rather smaller than usual, either on account of the relatively small amount of material available or because of the concentration of minor components in a small fraction. These fractions (6–12 g.) have been distilled through a Dixon 6 in.