The radioactivity was measured with a thin end-window Geiger-Müller counter on 0.5 ml. of the solution dried on a nickel planchette. Loss of radioactive cyanide or thiocyanate was prevented by adding excess AgNO_3 before drying and by using a hotplate in place of the more usual infrared lamp, which could cause some photolytic decomposition.

SUMMARY

1. The structures and stabilities of a series of cobalamins have been studied, mainly by tracer techniques and by measurements of absorption spectra and partition coefficients.

2. It has been shown that, besides the neutral and basic classes of cobalamins, there is a third class

of acidic cobalamins. They contain two acidic ions, which need not be the same, or one divalent ion like sulphite. They are unstable and only persist in solutions containing excess of the co-ordinating ion.

3. Using radioactive carbon and sulphur it was shown that the cyano group in vitamin B_{12} exchanges with inorganic cyanide, rapidly in alkaline solution but very slowly in acid. In high concentrations of thiocyanate in acid or alkaline, but not in neutral solutions, the cyano group is partly displaced by thiocyanate.

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Studies on the Composition of Horse Oil

2. THE COMPONENT FATTY ACIDS OF LIPIDS FROM FATTY TISSUES, MUSCLE AND LIVER

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In part 1 of this series (Brooker & Shorland, 1950) it was shown that the fats of pasture-fed animals differed considerably from species to species in fatty acid composition. Whereas linolenic (octadecatrienoic) acid, the chief component of the pasture fatty acids, comprised about 17 % of the depot fat of horses, it was found only in traces in the fats of pasture-fed sheep and oxen. Horse fats differed further from those of sheep and oxen by having greater proportions of hexadecenoic acid and smaller amounts of stearic acid. These results have since been generally confirmed by Gupta & Hilditch

(1951). In addition, Bruce & Shorland (1951) have shown in a preliminary communication that, as observed for other animals (cf. Hilditch & Shorland, 1937), the liver phospholipins of the horse contained more stearic and highly unsaturated C_{20-22} acids, but less palmitic and hexadecenoic acid, than did the liver glycerides. It was found, however, that although the respective contents of total C_{18} unsaturated acids and of oleic acid of the phospholipin and glyceride fractions of the horse liver were similar, the phospholipin contained about three times as much octadecadienoic acid but only one-

Table 1. *Characteristics of horse lipids used in this investigation*

	Lipid in tissues (%)	P (%)	N (%)	Unsaponifiable (%)	Cholesterol in lipid	
Combined (%)					Free (%)	
FAT HORSES						
Fatty tissues (whole fraction)	93.2	Trace	Trace	0.53	—	—
Muscle glycerides	9.3	0.21	—	2.73	—	—
Muscle phospholipins	0.3	3.93	—	—	—	—
Liver glycerides	2.7	0.53	0.44	18.5	1.40	8.65
Liver phospholipins	3.3	3.80	1.89	—	—	—
LEAN HORSES						
Fatty tissues (whole fraction)	80.1	Trace	Trace	0.20	—	—
Muscle glycerides	2.9	0.36	—	6.10	—	—
Muscle phospholipins	0.3	3.79	2.13	—	—	—
Liver glycerides	1.2	0.50	0.38	31.4	5.08	17.52
Liver phospholipins	3.3	3.58	1.77	—	—	—

sixth the amount of linolenic acid present in the glyceride. Such marked differences in composition between the C_{18} unsaturated acids of the glycerides and phospholipins of the same animal do not appear to have been previously recorded.

The purpose of the work now reported has been to examine the fatty acid composition of lipids from various tissues of the horse, particularly the distribution of the C_{18} unsaturated acid constituents.

EXPERIMENTAL

Preparation of material

The material was obtained as in the previous investigation (Brooker & Shorland, 1950) from horses killed at the Auckland Farmers' Freezing Company Works at Southdown, New Zealand. A batch of fifty-five horses was killed on the morning of 4 November 1947, yielding an average dressed weight of carcass of 721 lb. After chilling overnight the carcasses were used for sampling. In general, most of the animals contained insufficient readily available fatty tissue for study by the usual ester fractionation techniques. However, some 'fat' animals in contrast to the more typical 'lean' animals had a layer of external fatty tissue over the greater part of the body except for portions of the shoulder. In addition, the flare fat in the abdominal cavity was in places up to 5 cm. thick as compared with only a relatively thin layer in the lean horses. For the purpose of this study five typical lean horses and two fat horses were selected. The fatty tissues used were representative of the external fat as well as of the abdominal cavity, and for the lean horses it was necessary to take all the available material for the sample. The fat horses, however, contained more than 20 times as much readily available fat as the lean horses, so that a representative sample only was used. In the case of the muscle tissues a series of cuts was prepared comprising the silver-side, tender loin, sirloin, prime rib-cut, blade and thin-flank. The tissues were finely sliced and representative portions taken to make a composite sample which was heated in boiling 95% (v/v) ethanol for 20 min. and then stored in sealed jars.

The liver samples, total weights 12.7 and 27.6 kg., taken respectively from two fat and five lean carcasses selected

from another killing of sixty-eight horses (average dressed weight 734 lb.) on 7 November 1947, were treated and preserved as described above.

After removal of the ethanol and water *in vacuo* at 100° the tissues were extracted with light petroleum containing 10% ethanol (v/v). Following evaporation of the solvent on the water bath, under reduced pressure during the last stages, the lipids were taken up in approximately 10 vol. of light petroleum and washed with about 0.1 vol. of water three times to remove impurities. The aqueous layers after evaporation *in vacuo* were re-extracted with light petroleum, but yielded no appreciable amount of lipid.

Separation of phospholipins and glycerides

The separation of the phospholipins and glycerides was carried out in the usual way by dissolving the lipids as completely as possible in 9 vol. of boiling acetone and refluxing for 20 min. The mixture was then allowed to cool to room temperature (cf. Hilditch & Shorland, 1937). To remove the traces of phospholipins present in the crude glycerides, the acetone-soluble fractions were cooled to 0° in 9 vol. of the solvent, in the presence of a few drops of an ethanolic solution saturated with CaCl_2 . After decanting the supernatant liquid the small amount of precipitate was refluxed with acetone and allowed to cool, and the acetone solution again decanted to separate the acetone-insoluble material which was then added to the phospholipin fraction. Even though these processes were carried out repeatedly, the glyceride fractions could not be freed from phospholipin. Further purification (cf. Table 2) of the liver glycerides of the fat and lean horses, which contained respectively 16.8 and 14.5% of free fatty acids (calculated as oleic), was achieved by dissolving the fractions in ether and washing with 10% (w/v) K_2CO_3 solution.

In the case of fat horses, the crude glycerides of the muscle (98.0 g.) were dissolved in light petroleum (b.p. 40–70°) and chromatographed on a column 4.0 cm. in diameter packed with a mixture (300 g.) containing equal proportions by weight of finely ground MgO and CaCO_3 . The glyceride fraction (85.96 g.) obtained by repeated elution with light petroleum showed no detectable amount of P but contained 0.45% of free fatty acids expressed as oleic acid. The adsorbed material (11.38 g.; 1.62% P) recovered by extraction with ether after acidification of the

adsorbent with dilute HCl consisted of 56.8% free fatty acids (expressed as oleic) and phospholipins. Further treatment with acetone according to the procedure outlined above yielded an acetone-insoluble fraction (3.89 g., 3.15% P).

Ester fractionation analyses

These were carried out as previously described by Brooker & Shorland (1950). In the case of the phospholipins, however, the fractions were first submitted to ethanolysis by boiling for 3 hr. under reflux with 3 vol. of ethanol containing 1% (w/v) H_2SO_4 before saponification with ethanolic potash in the usual way.

The ester fractionation analyses of liver glycerides were based on the purified fractions (cf. Table 2) from which the unsaponifiable matter was removed by thorough extraction of the aqueous ethanolic soap solutions with ether.

Since fatty acid analyses of the crude and purified fractions of the muscle glycerides of the fat horses, based respectively on 76.8 and 134.8 g. of material, showed no appreciable differences (cf. Table 3) within the limits of the accuracy of the method, the glyceride fraction from the muscle tissue of the lean horses was not further purified, and the results,

shown in Table 5, for the muscle tissue glycerides of both the fat and the lean horses were based on the unpurified fractions.

The amounts of muscle phospholipins were too small for an accurate ester fractionation analysis. Accordingly, the fatty acids were separated into 'solids' and 'liquids' as described by Brooker & Shorland (1950) and the characteristics of the fractions determined as shown in Table 4, which includes, for comparison, data obtained for the liver phospholipins.

Composition of the C_{18} unsaturated acids

The composition of the C_{18} unsaturated acids was studied separately by applying the alkali-isomerization method of Hilditch, Patel & Riley (1951) to purified concentrates of C_{18} unsaturated acids obtained during the course of ester fractionation analyses. Although the muscle phospholipins, as stated above, were not available in sufficient quantity for an accurate ester fractionation analysis, it was found practicable to prepare, by distillation of the methyl esters of the liquids, a C_{18} fraction for analyses by the alkali-isomerization method.

Table 2. *Purification of the crude liver-glyceride fractions*

Purified glyceride		Ether-soluble fractions from acidified K_2CO_3 washings			
		Acetone-soluble free fatty acids		Acetone-insoluble phospholipins	
% of original fraction	P (%)	% of original fraction	P (%)	% of original fraction	P (%)
FAT HORSES					
71.3	0.13	21.7	0.72	7.0	3.60
LEAN HORSES					
77.1	0.14	15.1	0.91	7.8	2.75

Table 3. *Comparison between the fatty acid composition of the crude and purified glycerides of muscle tissue of fat horses*

	Fatty acids (wt. %)									
	Saturated					Unsaturated				
	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{14}	C_{16}	C_{18}	C_{20}	
Crude glyceride	0.4	2.4	29.9	3.0	0.2	1.5 (-2.0H)	8.7 (-2.0H)	50.8 (-3.2H)	3.1 (-4.4H)	
Purified glyceride	—	3.9	30.4	2.5	—	1.6 (-2.0H)	9.4 (-2.0H)	48.8 (-3.2H)	3.4 (-3.9H)	

Table 4. *Characteristics of muscle and liver phospholipin fatty acid fractions*

Fraction	Solids			Liquids		
	% of total	Saponification equivalent	Iodine value	% of total	Saponification equivalent	Iodine value
FAT HORSES						
Acetone-insoluble from muscle lipids	32.6	284.2	19.7	67.4	289.9	168.8
Phospholipin recovered from glyceride fraction of muscle lipids	39.9	276.9	16.9	60.1	274.9	145.4
Liver phospholipins	33.4	280.2	3.8	66.6	284.1	147.8
LEAN HORSES						
Acetone-insoluble from muscle lipids	34.4	286.6	19.2	65.6	281.9	117.3
Liver phospholipins	38.3	284.6	7.1	61.7	283.4	163.3

Owing to the extreme differences between the glycerides and phospholipins in respect of their C_{18} unsaturated acid compositions (Table 6) it was found advantageous to allow for the traces of contaminating phospholipins in the glyceride fractions as outlined by Hilditch & Shorland (1937). For example, the muscle glycerides of the fat horses showed a difference between the uncorrected value (8.5%) for octadecadienoic of 1.9 units % acid content and the corrected value (6.6%), as compared with the value of about ± 0.4 unit % for the standard deviation of the method.

DISCUSSION

Hilditch & Shorland (1937), in their work on the liver lipids of the ox, sheep and pig, indicated that the ester fractionation analyses of the glycerides separated from phospholipins with acetone may be somewhat in error unless a correction is made for the invariable presence of small proportions of contaminating phospholipin fatty acids. In the present work, to avoid possible errors owing to phospholipin contamination, the liver glycerides from the acetone separation were freed from all but traces of phospholipins and free fatty acids by alkali refining, while the accuracy of the fatty acid composition analyses of the muscle glycerides was confirmed by comparing the results obtained before and after purification by chromatographic adsorption.

A separate study of the C_{18} unsaturated acids by means of alkali-isomerization technique showed such extreme differences in composition between the phospholipin and glyceride fractions of the same tissues that it was found advantageous to allow for the traces of phospholipins in the glyceride fractions, and the results in Table 6 are based on the values which have been corrected according to the procedure outlined by Hilditch & Shorland (1937).

Phospholipin fractions when precipitated from acetone solution may sometimes be contaminated with glycerides of one unsaturated and two saturated acids or of three saturated acids (Hilditch & Shorland, 1937). Fortunately not more than traces of such glycerides were encountered in the present work.

Fatty acid composition of the glycerides and phospholipins

Although, from the precautions taken, as outlined above, it would appear that the glyceride fractions shown in Table 6 are strictly comparable, there still remains the possibility that the presence of cholesterol esters may affect the apparent fatty acid composition of the liver glycerides. Kelsey & Longenecker (1941) found differences in fatty acid composition between the neutral glycerides and

Table 5. *Fatty acid composition of the lipid fractions from the fatty tissues, muscle and liver of the horse. (Fatty acids wt. %)*

	Saturated					Unsaturated				
	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
FAT HORSES										
Fatty tissue glycerides	—	2.4	29.7	4.3	0.2	1.4 (-2.0H)	6.5 (-2.0H)	52.4 (-3.4H)	3.1 (-3.3H)	—
Muscle glycerides	0.4	2.4	29.9	3.0	0.2	1.5 (-2.0H)	8.7 (-2.0H)	50.8 (-3.2H)	3.1 (-4.4H)	—
Liver glycerides	—	1.7	25.2	5.3	—	1.6 (-2.0H)	8.3 (-2.0H)	52.8 (-4.5H)	3.4 (-6.2H)	1.7 (-6.0H)
Liver free fatty acids	—	—	10.5	20.6	0.5	—	9.5 (-2.0H)	48.7 (-3.8H)	5.9 (-6.0H)	4.3 (-6.0H)
Liver phospholipins	—	—	10.8	20.7	4.6	—	2.0 (-2.0H)	50.6 (-3.4H)	10.4 (-5.7H)	0.9 (-5.7H)
LEAN HORSES										
Fatty tissue glycerides	—	3.2	26.2	5.6	0.3	0.9 (-2.0H)	4.6 (-2.0H)	56.3 (-3.5H)	2.9 (-4.1H)	—
Muscle glycerides	—	1.7	26.8	5.1	—	0.8 (-2.0H)	7.9 (-2.0H)	54.5 (-3.3H)	3.2 (-4.8H)	—
Liver glycerides	—	0.7	22.5	10.8	—	0.6 (-2.0H)	6.1 (-2.0H)	49.7 (-3.5H)	9.6 (-6.0H)	—
Liver free fatty acids	33.4					—	10.9 (-2.0H)	45.1 (-3.4H)	10.6 (-3.4H)	—
Liver phospholipins	—	—	11.1	18.2	6.5	—	3.4 (-2.0H)	48.6 (-3.3H)	9.7 (-4.5H)	2.5 (-4.5H)
Bulk sample of bone oil (Brooker & Shorland, 1950)	0.2	2.8	25.2	4.6	—	0.7 (-2.0H)	8.1 (-2.0H)	57.4 (-3.5H)	1.0 (-4.0H)	—
Australian horse oil	—	2.0	24.0	7.0	—	0.6 (-2.0H)	7.3 (-2.0H)	55.5 (-3.5H)	3.6 (-3.5H)	—

cholesterol esters of blood plasma. The amounts of combined cholesterol in the liver glycerides of the fat and lean horses, 1.40 and 5.08 % respectively, however, are not sufficient to affect appreciably the interpretation of the results.

From Table 5 it will be seen that, while all the glyceride fractions are generally similar in fatty acid composition, the liver glycerides differ from those of the muscle and fatty tissues in containing slightly less (about 4 %) palmitic acid and rather more C_{20-22} highly unsaturated acids. In addition, the liver glycerides of the lean horses are shown to contain approximately twice as much stearic acid as is present in the remaining glycerides. The absence of increased proportions of hexadecenoic acid in the liver glycerides of the horses as compared with the depot fats, distinguishes horses from sheep, oxen and pigs, where the glycerides show considerably more hexadecenoic acid than is found in the depot fats (Hilditch & Shorland, 1937). A somewhat higher proportion (3 units %) of palmitic acid is maintained in the glyceride fractions of the fat horses as compared with the corresponding fractions of the lean horses. The content of stearic acid is uniformly low (3.0–5.6 %) in all the glycerides studied except the liver glycerides of the lean horse in which it rises to 10.8 % and in which, also, the amount of C_{20} unsaturated acids increases to 9.6 %, or about three times the amount found in the remaining glycerides.

Included for comparison with the present results are the bulk sample of bone oil previously studied by Brooker & Shorland (1950), and a sample of Australian horse oil obtained by steam rendering of horses from Queensland. These analyses, together with those of previous workers summarized by Gupta & Hilditch (1951) show that the horse glycerides, irrespective of the particular fatty depot or the locality from which the sample was taken, have a generally similar fatty acid composition.

Horse fats in general resemble those of other animals in regard to their content of palmitic acid (25–30 %), but as compared with the depot fats of the ox, sheep or pig (cf. Hilditch, 1947), they contain considerably less stearic acid (3–7 % as compared with 10–30 %) but more hexadecenoic acid (4.6–8.7 % as compared with 1–3 %) and appreciable proportions (about 3 %) of C_{20-22} unsaturated acids, as compared with only trace amounts (1 % or less) found in depot fats of these animals.

The fatty acid analyses of the phospholipins accord with the previous work of Hilditch & Shorland (1937) on the liver lipids of oxen, sheep and pigs in showing, as compared with the corresponding glycerides, increased proportions of stearic acid and C_{20-22} highly unsaturated acids, together with reduced proportions of palmitic and hexadecenoic acids. The similarity in fatty acid composition

between the free fatty acid and phospholipin fractions of the liver lipids, suggests that the former fraction may have originated mostly from the latter. This view is also supported by comparing the composition of the two C_{18} unsaturated acid fractions, as shown in Table 6. From previous investigations (cf. Shorland, 1948) it has been found that by taking special precautions, a low content of free fatty acids may be obtained, indicating that the amounts present in living tissue are very low indeed. Although, as previously indicated, no ester fractionation analyses were made on the muscle phospholipins because only small amounts of material were available, the data given in Table 3 suggest their fatty acid composition resembles that of the liver phospholipins.

The C_{18} unsaturated acids

Distribution of the C_{18} unsaturated acids in the glyceride and phospholipins. The close agreement between the ester fractionation analyses of all the glyceride fractions (cf. Table 5) could be taken as proof of similarity in fatty acid composition. However, it is clear from Table 6 that such is not the case, and that the C_{18} unsaturated acids of glycerides from the fatty tissues, muscle and the liver, though similar as regards their total proportions and mean unsaturations, have quite different compositions in respect to the monoene, diene and triene constituents. From Table 6 it will be seen that a similar pattern in regard to the distribution of the C_{18} unsaturated acids is followed in the corresponding glycerides of the fat and of the lean horses. The proportions of glyceride oleic acid are similar (about 60 %) in the muscle and fatty tissues, being slightly greater in the former, but are reduced in the liver to approximately half the value found in the other tissues. The proportions of glyceride diene show a threefold increase in passing from the fatty tissues to the liver, with the muscle tissues showing an intermediate value. The glyceride linolenic acid is present in greatest proportions in the liver where it comprises the main C_{18} unsaturated acid constituent. The fatty tissue glycerides are shown to contain slightly less of this constituent and the muscle glycerides least. Linolenic acid is shown to be the least variable constituent and octadecadienoic acid the most variable of the C_{18} unsaturated acid constituents of the glycerides.

According to Callow's (1935) theory, fast-growing fatty depots, as compared with those that grow more slowly, contain more of the fat synthesized by the animal and less of the fat derived by direct assimilation. In agreement with this theory the fatty tissue and muscle glycerides of the fat horses are shown to contain less of the dietary octadecadienoic and linolenic acids than the lean horses. In the liver glycerides, however, the reverse is true.

Table 6. *Composition of the C₁₈ unsaturated acids expressed as a wt. % of the C₁₈ unsaturated acids*

(Figures in brackets indicate wt. % expressed on the total fatty acids. (tr., trace.))

Fraction	Conjugated		Non-conjugated		Oleic (by difference)	Triene
	Diene	Triene	Diene	Triene		Diene
FAT HORSES						
Fatty tissue glycerides	1.0 (0.5)	tr. (tr.)	6.3 (3.3)	30.8 (16.1)	61.9 (32.5)	4.22
Muscle glycerides	0.1 (tr.)	tr. (tr.)	6.6 (3.4)	24.6 (12.5)	68.7 (34.9)	3.67
Muscle phospholipins*	0.4	0.1	66.8	8.1	24.6	0.12
Muscle phospholipins†	0.5	0.1	54.7	9.4	35.3	0.17
Liver glycerides	0.7 (0.4)	0.1 (tr.)	19.0 (10.0)	46.9 (24.8)	33.3 (17.6)	2.39
Liver free fatty acids	1.0 (0.5)	0.1 (tr.)	37.0 (18.1)	28.2 (13.7)	33.7 (16.4)	0.75
Liver phospholipins	1.2 (0.6)	0.2 (0.1)	59.5 (30.1)	7.8 (4.0)	31.3 (15.8)	0.13
LEAN HORSES						
Fatty tissue glycerides	1.3 (0.7)	tr. (tr.)	7.0 (3.9)	35.4 (20.0)	56.3 (31.7)	4.27
Muscle glycerides	0.3 0.2	tr. tr.	11.1 (6.0)	27.0 (14.7)	61.6 (33.6)	2.37
Muscle phospholipins	2.3	0.4	40.4	23.6	33.3	0.56
Liver glycerides	1.9 (0.9)	0.4 (0.2)	16.9 (8.4)	40.4 (20.1)	40.4 (20.1)	2.17
Liver free fatty acids	2.0 (0.9)	0.3 (0.1)	34.8 (15.7)	15.1 (6.8)	47.8 (21.6)	0.42
Liver phospholipins	4.9 (2.4)	0.2 (0.1)	55.2 (26.8)	7.3 (3.5)	32.4 (15.8)	0.12

* Acetone-insoluble fraction from total lipid.

† Recovered from crude glyceride fraction.

Characteristic of the lipids of the lean horses is the somewhat higher level of conjugated diene acid, particularly in the liver phospholipins where it comprises 4.9 % of the total C₁₈ unsaturated acid or approximately four times the amount present in the lipids of the fat horses.

From Table 6 it will be seen that the distribution of the C₁₈ unsaturated acids follows a similar pattern in all the phospholipins studied. The main component is octadecadienoic acid (40.4–66.8 %) followed by oleic acid (24.6–40.4 %), with linolenic acid (7.3–23.6 %) as the minor constituent. As compared with the glycerides, the phospholipins are shown to contain more octadecadienoic acid, but less linolenic acid as may be judged from their respective triene/diene ratios of (2.17–4.27) and (0.12–0.56). The C₁₈ unsaturated acids of the phospholipins studied show about half the amount of oleic acid found in the glycerides of the fatty tissue and the muscle, but are comparable in this respect with the liver glycerides.

The relationship between the C₁₈ triene/diene ratio of the dietary fat and of the depot fat

The diene and triene C₁₈ unsaturated acids found in the depot fats of animals are apparently not synthesized in the body, but are derived by direct

assimilation from the diet (Hilditch, Lea & Pedelty, 1939; Shorland & de la Mare, 1945). In agreement with unpublished results for the C₁₈ triene/diene ratio of approximately 4.0 found for typical New Zealand pasture, the C₁₈ triene/diene ratios of the main depot fats of pasture-fed horses (Table 6) are shown to vary from 2.37 to 4.27. Similar values for fats of pasture-fed horses have also been obtained by other workers (Hilditch & Jaspersen, 1944; Brooker & Shorland, 1950; Gupta & Hilditch, 1951).

The lower C₁₈ triene/diene ratio (0.1–0.4) observed by Holmberg & Rosenqvist (1949) for the bone oils and kidney fats of horses fed oats and hay, accords with the fact that in oat lipids linoleic and not linolenic acid is the main highly unsaturated C₁₈ constituent (see Hilditch, 1947). Similarly, the lower ratio of 1.02 found in the present work for the Australian horse oil could well be due to the very different types of forage plants in the Queensland tropics as compared with those in New Zealand or Great Britain.

The evidence suggests that the ratio of C₁₈ triene/diene acids present in the diet remains essentially unchanged during the passage of the dietary fat through the horse, and its ultimate incorporation in the fatty depots. In contrast, pasture-fed sheep and oxen neither readily incorporate the dietary

linolenic and octadecadienoic acids in their fatty depots (Shorland, 1950) nor store such amounts of these dietary acids as are retained in the ratio in which they are present in the diet. Unpublished work in this laboratory, for example, indicates that the main fatty depots of pasture-fed sheep and oxen show a C_{18} triene/diene ratio of 0.3–0.4.

Although the main fatty depots of the horse show a C_{18} triene/diene ratio not unlike that of the pasture lipids, there is, nevertheless, some variation in the composition of the C_{18} unsaturated acids between the different fatty depots, and especially between the phospholipin and glyceride fractions (cf. Table 6). Such changes in the C_{18} triene/diene ratios could be brought about by selective transformation of octadecadienoic acid and linolenic acid, which, according to Widmer & Holman (1950), are converted by the animal to arachidonic and C_{22} hexaene acids respectively. Though some such transformation has occurred in the horse, the proportions of C_{20-22} highly unsaturated acids in the depots, as compared with those of octadecadienoic and linolenic acid, are very small indeed. Selective oxidation of the diene and triene C_{18} constituents could also be a factor in changing the C_{18} triene/diene ratio, but the presence of similar proportions of octadecadienoic acid in the depot fats of animals before and after a period of starvation (Longenecker, 1939; Hilditch & Pedelty, 1941*a, b*) may well indicate a comparative stability of the more highly unsaturated acids in contrast to the rapid turnover of oleic, stearic and palmitic acids postulated by Schoenheimer & Rittenberg (1936) and by Rittenberg & Schoenheimer (1937).

It is therefore concluded that selective deposition of the fatty acids during the transport of the fatty substances between the liver and the fatty depots, and vice versa, rather than selective transformation or oxidation, is the main factor responsible for the observed differences in composition of the C_{18} unsaturated acids between the fatty depots of the different tissues, and between the phospholipins and the glycerides of the same tissues of the horse.

*The mechanism of fat deposition in the horse
as indicated from the glyceride structure*

From the principle of even distribution (Hilditch, 1947) the presence of some 60 % of linolenic acid in the glyceride fraction of pasture lipid implies the occurrence of this acid mainly in the form of dilinolenotriglycerides. In the depot fat of the horse, however, it has been shown by Gupta & Hilditch (1951) that linolenic acid is present almost entirely as a monolinolenotriglyceride. Even allowing for some enzyme action during ingestion of the pasture (cf. Shorland, 1944) with the production of some free fatty acids, the difference in glyceride structure between the dietary fat and the depot fat does not

afford evidence in favour of Frazer's (1946) theory implying the absorption of some of the fat directly without conversion into free fatty acid. The evidence of the glyceride structure rather points, however, to the absorption of the dietary fat as free fatty acids, and in part, perhaps, as monoglycerides (cf. Reiser, Bryson, Carr & Kuiken, 1951) which are subsequently selectively distributed and mixed in with the fatty acids synthesized by the animal to form new glycerides in the various fatty depots of the horse.

SUMMARY

1. The fatty acid composition of the glycerides of the fatty tissues, muscle and liver of the horse as determined by ester fractionation were shown to be generally similar and to contain 25–30 % palmitic acid, 3–6 % stearic acid, 4–8 % hexadecenoic acid, 50–55 % C_{18} unsaturated acids, and minor proportions of myristic, tetradecenoic and C_{20-22} unsaturated acids.

2. A separate examination of the C_{18} unsaturated acids showed considerable differences in composition between the different glycerides. The liver glycerides, in particular, contained less oleic acid than the glycerides of the muscle and fatty tissues (33.3–40.4 as compared with 56.3–68.7 %) and the ratio C_{18} triene/diene showed a progressive diminution from 4.22–4.27 to 2.17–2.39 in passing from the glycerides of the fatty tissues to those of the muscle and liver.

3. The liver phospholipins of the horse as compared with the liver glycerides were shown, in conformity with the observations by other workers on a range of different animals, to contain more stearic acid and C_{20-22} unsaturated acids but less palmitic and hexadecenoic acid. In addition, the liver phospholipins contained more octadecadienoic acid (26.8–30.1 as compared with 8.4–10.0 %) but less linolenic acid (3.5–4.0 as compared with 20.1–24.8 %) than did the liver glycerides.

4. The composition of the C_{18} unsaturated acids in the muscle phospholipins was found to be similar to that of the liver phospholipins.

5. The distribution pattern of the C_{18} unsaturated acids together with the nature of the glyceride structure in the fatty depots of pasture-fed horses compared with that of the fats in pasture suggests that the dietary fats of the horse cannot be directly absorbed as such, but that the fatty acids of the diet are mixed in with those synthesized by the animal, and selectively redistributed to form new glycerides.

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Studies in Biochemical Adaptation. The Origin of Urinary Ammonia as Indicated by the Effect of Chronic Acidosis and Alkalosis on Some Renal Enzymes in the Rat

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The origin of urinary ammonia, which is an important part of the mechanism for maintaining the acid-base equilibrium, has been the subject of much study. The early view was that it was derived from urea (Barnett & Addis, 1917; Nash & Benedict, 1921; Bollman & Mann, 1930). More recent work has shown this view to be incorrect (Van Slyke *et al.* 1943; Pitts, 1936). It is now believed that the ammonia is derived from glutamine and from other amino-acids. Whereas the kidney contains no urease, it does contain glutaminase and a variety of deaminating and transaminating enzymes acting upon amino-acids (Krebs, 1935a, b; Blanchard, Green, Nocito & Ratner, 1944; Mylon & Heller, 1948).

Support for this is found from two types of experiments with dogs *in vivo*. In the first, possible precursors of urinary ammonia were administered intravenously, and the effect on ammonia excretion

determined by estimation of ammonia either in the urine or in the renal artery and vein. By such experiments, Polonovski & Boulanger & Bizard (1934), Polonovski & Boulanger (1938), Bliss (1941) and Lotspeich & Pitts (1947) suggest that glycine, D- or L-alanine, D- or L-leucine and DL-aspartic acid may act as precursors of urinary ammonia, whereas L-arginine, L-lysine and L-glutamic acid apparently do not. Such results are not conclusive evidence of the normal mechanism in the living organism. This is obvious in regard to the unnatural (D) forms of amino-acids which are certainly not the normal precursors, though their administration increases the excretion of urinary ammonia. It may also be true of the natural acids, since they do not normally occur in the high concentrations which result from the intravenous injections used in the experiments.

In the second type of *in vivo* experiment, estimations were made of the rate of removal from the blood of the possible precursors themselves, already present in the blood. In this way, Van Slyke *et al.* (1943) showed that L-glutamine was removed from the renal blood in amounts which account for 60 %

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