VIII. THE FATTY ACIDS OF THE CAT'S KIDNEY. I.

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Introduction.

When the fatty acids which can be isolated from the cat's kidney are compared with those from the kidneys of other animals it is found that they present some striking differences. Whereas the mixed fatty acids from sheep or ox kidneys amount to about 2 % of the weight of the fresh organ and possess an iodine value of approximately 135, cat's kidneys yield about 4 % of fatty acids with an iodine value in the neighbourhood of 60. In spite of their lower iodine value, however, these mixed acids are semi-liquid whilst the more unsaturated acids from other animals are quite solid at room temperatures.

The earliest recorded values are those of Leathes and Meyer-Wedell [1909]. They found that the iodine values of fatty acids from the kidneys of normal cats were from 60 to 70, almost identical, that is, with the iodine value of 65 for the fatty acids from the adipose tissue fat. Mottram [1916] found that the average amount of fat in the kidneys of eight well-fed healthy cats was 5·11 % with an average iodine value for the fatty acids of 56, actually lower than the average iodine value of 61·4 for the adipose tissue fatty acids from the same cats. He also observed that the kidney tubules were heavily lined with stainable fat. Mottram suggested that the abnormally low iodine values were the result of an infiltration of fat into the kidneys, but for reasons which will be discussed later it seems improbable that this can be the correct explanation.

The kidney fat of the cat has been re-investigated with the results which are now to be described.

Experimental.

Fatty acid estimations.

The low iodine values reported by Leathes and Meyer-Wedell [1909] and Mottram [1916] have been confirmed. It should be noted that Mottram's values refer only to the cortex of the kidney, but the following results show that the same is true for the whole kidney. The percentage of fatty acids in the kidneys was determined by the method described by Leathes and Raper 1925] and the iodine values were determined by the method of Wijs. Each

value given was obtained from the whole of one kidney; where two values are given they refer to left and right kidneys respectively. The iodine values were determined immediately after isolation of the fatty acids.

Table I.

Fatty acids (%)	Iodine value	Fatty acids (%)	Iodine value
3·72 3·69	82·6	6·37 6·83	71·7
4·28 4·61	84·1	5·70 5·48	71·0
5·40 6·54	69·3	3·65 3·78	87·7
4·14 4·37	80·5	3·21	97·8
6·77 6·73	69·6	5·68	76·0
5·58	63·5	2·75	113·0
5·63 5·92	69·5	2·16	98·0
6·30 6·18	88·7	4·15	79·0
5·29 4·76	79·9	3·58	87·4
4·07 4·06	86·5	6·83	53·2

Fatty acids: Max. value = 6·83 %.
Min. value = 2·16 %.
Mean of 32 = 4·9 %.

These values may be compared with those from typical beef kidney which had 2·09 % and 2·02 % fatty acids with corresponding iodine values of 133·8 and 131·0; and with the average for 34 human kidneys examined by Imrie [1914] of 1·9 % with iodine value 131.

Examination of the Fatty Acids.

A. *The isolation of the mixed fatty acids.*

The kidneys were collected from a large number of cats during a period of several months. Immediately after death the kidneys were removed from the body, carefully freed from all visible fat and connective tissue, weighed, cut into slices and placed in a small porcelain basin. The tissue was then saponified with a 50 % aqueous solution of caustic potash until completely liquid, methylated spirit was then added and heating continued until hydrolysis was complete. The resulting soap solution was poured into a stock-bottle. When about a kilogram of kidneys had been collected and saponified in this way, the accumulated soap solution was made strongly acid with 40 % sulphuric acid. After dilution with water to a suitable volume it was extracted three times with light petroleum. The extracts, which were deep red in colour, were collected in a flask and the solvent was removed on the water-bath in a slow current of carbon dioxide, the last traces being removed *in vacuo*. The crude fatty acids remaining in the flask were freed from unsaponifiable matter as completely as possible by repeatedly shaking out their solution in potassium hydroxide with light petroleum. The aqueous fluids were again acidified with sulphuric acid and extracted with light petroleum, the fatty acids being recovered by evaporation of the dried extract in a stream of carbon dioxide to prevent oxidation.

The mixed fatty acids formed a semi-liquid mass, yellow-brown in colour. If the flask containing them was left for some days in a tilted position, a liquid portion drained away from the solid acids. The whole readily liquefied

on very gentle warming. A peculiar musty smell was invariably associated with the mixed acids, though this may have been due to the presence of small amounts of impurities. The mixed acids usually had an iodine value about 56 and M.W. by titration about 274.

B. *Separation of the mixed fatty acids.*

The mixed fatty acids were separated into a liquid and a solid fraction by means of the lead soap-ether process.

The results of three distinct separations are summarised in Table II.

Table II. *Results of the separation of the fatty acids by the lead soap-ether process.*

	Mixed acids		Unsaturated acids			Saturated acids	
Batch	Iodine value	M.W.	Iodine value	M.W.	% of mixed	Iodine value	M.W.
A	56·9	264	62·5	263	61·5	4·4	—
B	56·4	274	67·3	274·2	60·6	5·8	276·4
C	56·4	274	68·7	282·4	63·9	14·0	275

The solid fraction (lead soaps insoluble in ether). These fatty acids were quite hard and solid, looking exactly like stearic acid. The M.W. of 274 combined with the very low iodine value suggests that they are mainly a mixture of palmitic and stearic acids in about equivalent proportions.

This is confirmed by the fact that an acid was obtained by repeated crystallisation of these acids from alcohol which had iodine value 0, M.W. 283·6 and M.P. 68·5°. 0·2280 g. acid neutralised 8·04 cc. $N/10$ NaOH; equivalent wt., 283·6. Stearic acid requires equivalent wt. 284, M.P. 69·2°, iodine value 0.

The liquid fraction (lead soaps soluble in ether). The acids composing this fraction were quite liquid even after standing for many months, although the iodine value was only 67·5. They were deep red in colour, but by boiling with charcoal in light petroleum solution they were finally obtained as a pale yellow oil. The peculiar musty odour was still apparent.

C. *Further investigation of the liquid fraction.*

The fluidity of this fraction combined with the very low iodine value (67·5) suggested the possible presence in the mixture of a liquid saturated acid. If this view were correct it should be theoretically possible to separate it from the liquid unsaturated acids by a preliminary hydrogenation of the mixture, by which these latter acids would be turned into solid saturated acids, followed by the usual lead soap-ether separation.

Hydrogenation of the liquid fraction. The hydrogenation was effected by the use of a platinum catalyst prepared by the method of Voorhees and Adams [1922]. This was found to be superior to a palladium hydrosol prepared by the method of Paal [1902, 1904]. Before the catalyst can be used for hydrogenations it must first be converted from the finely divided platinum

oxide into finely divided platinum black. A small quantity of the platinum oxide catalyst is shaken into a suspension in alcohol in a small round flask, the air is then swept out by a current of hydrogen from a Kipp's apparatus, and the suspension shaken in an atmosphere of hydrogen. In a short time a perfectly black, fine suspension is obtained. The fatty acids dissolved in a small volume of alcohol are then added to the flask containing the platinum-black catalyst and shaken for 2–3 hours in a hydrogen atmosphere under slightly more than atmospheric pressure. 26 g. of mixed liquid acids (iodine value 67·5) were in this way converted into 25 g. of hydrogenated acids which had the iodine value 7·8 and were quite solid.

Separation of the hydrogenated acids. The 25 g. of hydrogenated acids were next separated by the lead soap-ether process into (1) *a solid fraction*—a white hard solid, M.P. 69°, iodine value 0, M.W. 283·9, which therefore consists of pure stearic acid formed by hydrogenation of oleic and linoleic acids present in the liquid acids, and (2) *a liquid fraction*—7·5 g. of pale yellow mobile oil, having the iodine value 11·3, M.W. 268.

Esterification of the liquid acids. The 7·0 g. of liquid acids were dissolved in 50 cc. of methyl alcohol containing 3 cc. of sulphuric acid and heated on a water-bath for 2 hours. The liquid was then poured into a large excess of water and the esters were shaken out with ether. The ethereal extract was washed with water, then with potassium carbonate solution, again with water and finally dried over calcium chloride. Removal of the ether left 6·8 g. of a pale yellow, almost odourless, mobile oil.

The methyl esters were then distilled under a pressure of 15–20 mm. The first fraction distilled mainly at 105–115°, the temperature then rose fairly rapidly to 135° and the remainder of the esters distilled at 135–140°. The distillates were colourless faintly fatty-smelling oils. A dark brown, very viscous oil remained as a residue in the distilling flask, which after boiling for 2 hours with 20 % alcoholic potash remained unchanged. It was soluble in hot alcohol but insoluble in light petroleum or water.

Saponification of the ester fractions. The ester fractions were hydrolysed separately by boiling for 1–2 hours with 10 % alcoholic caustic potash. The resulting soap solutions were then acidified with H_2SO_4 and shaken out with light petroleum, and the extract was washed with water and dried. The fatty acids obtained on removing the solvent were from each fraction colourless, odourless, mobile oils.

Fraction (1). Wt. 1·2 g.

0·1938 g. neutralised 8·55 cc. N/10 NaOH. Equivalent wt. = 226·6.

0·1900 g. absorbed 0·2 mg. I_2. Iodine value = 0.

$C_{14}H_{28}O_2$ requires equivalent wt. 228.

Fraction (2). Wt. 1·98.

0·1506 g. neutralised 6·14 cc. N/10 NaOH. Equivalent wt. = 245·3.

0·1322 g. absorbed 0·5 mg. I_2. Iodine value = 0.

$C_{15}H_{30}O_2$ requires equivalent wt. 242.

0·16 g. of this acid dissolved in 10 cc. pure ethyl alcohol showed no optical activity.

The first fraction of these acids remained quite liquid when kept at a temperature of 0° for several days. The viscosity was rather higher than at room temperature but there was no sign of crystallisation. The higher boiling fraction on cooling to 0° deposited white, waxy crystals but the acids as a whole did not solidify. It seems that one acid crystallised out whilst another remained liquid. This fact, together with the knowledge that no C_{15}-acid is definitely known to be present in nature, suggests that the second fraction is probably a mixture of almost equal quantities of C_{14}- and C_{16}-acids. The C_{16}-acid would presumably be just solid at room temperatures.

D. *Oxidation of the fatty acids from the cat's kidney.*

The oxidation was carried out by two methods:

(1) *Hilditch's* [1926] *method.* Oxidation of the mixed acids by means of hydrogen peroxide (90/100 vols.) in glacial acetic acid at 70° as described by Hilditch gave rise to a dihydroxystearic acid, M.P. 92°. No sativic acid fraction was obtained.

(2) *Dilute alkaline potassium permanganate.* The mixed acids (iodine value 48) were also oxidised by dilute, ice-cold potassium permanganate in alkaline solution as was done by Hartley [1907] with acids from the liver.

Acids soluble in light petroleum formed 48 % of the original mixed acids. The iodine value was 27·3.

An acid insoluble in light petroleum, soluble in ether was obtained. This had M.P. 131° and was therefore dihydroxystearic acid.

An acid insoluble in light petroleum and ether, but soluble in boiling water, from which it crystallised on cooling, was also found. It had M.P. 164–166° corresponding with tetrahydroxystearic acid.

These two acids are derived from oleic and linoleic acids respectively. The presence of the latter acids in the fatty acids from the cat's kidney is therefore demonstrated.

E. *Bromination of the acids from the cat's kidney.*

1·38 g. of the liquid acid fraction (iodine value 67·5) were dissolved in 30 cc. of ether, and a 1 % solution of bromine in ether was added drop by drop to the ice-cold solution until excess of bromine was present. The solution was allowed to stand overnight in ice. A small white deposit was obtained which was filtered off through a weighed Jena glass crucible.

The ether was removed from the filtrate and the residue after weighing was treated with light petroleum (B.P. 60–80°). A dark brown precipitate was obtained; this was likewise filtered off and weighed.

1·38 g. of mixed liquid acids gave 1·85 g. bromination products, 0·02 g. insoluble in ether, 0·11 g. insoluble in light petroleum (B.P. 60–80°) and 1·72 g. of material (by difference) soluble in light petroleum.

Rollett [1909] has shown that about half of the linoleic tetrabromides produced from ordinary linoleic acid are soluble in light petroleum. Allowing for this, about 0·25 g. of the bromides out of the 1·85 g. are tetra- or poly-bromides. When it is considered that about 60 % of the weight of a tetra-bromide is bromine, it is obvious that the amount of acids more unsaturated than oleic acid in the 1·38 g. of mixed acids must be very small. It is safe to conclude therefore that nearly all the iodine absorption of the liquid acid fraction is due to the presence therein of oleic acid.

The lipoid fractions of cat's kidney.

A number of cat's kidneys were first exhaustively extracted with acetone and the residue was then extracted with ether. The two lipoid fractions obtained correspond approximately with the glycerides and the phospholipins respectively. For comparative purposes a similar treatment was given to a quantity of beef kidney and the results of the investigations on these fractions are summarised in Tables III–V.

Table III. *Appearance of extracts.*

Fractions	Cat	Beef
(1) Acetone extract	Deep orange viscous oil	Deep orange wax
(2) Ether extract	Pale orange semi-solid	Pale orange waxy extract
1*a*. Fatty acids from saponification of Fraction (1)	Liquid	Solid
2*a*. Same from Fraction (2)	Waxy solid	Solid

Table IV. *Distribution of lipins.*

Fraction	Cat kidney (1) %	Cat kidney (2) %	Beef kidney %
Acetone extract	3·01	1·85	0·87
Ether extract	1·99	2·00	1·26

Table V. *Iodine values.*

Fraction	Cat kidney (1) %	Cat kidney (2) %	Beef kidney %
Acetone extract:			
Before saponification	55·0	69·3	117·9
Fatty acids from saponification	56·7	63·9	130·2
Ether extract:			
Before saponification	67·2	59·8	84·0
Fatty acids from saponification	74·9	55·5	95·2

Discussion.

Mottram [1916] considered that the abnormally low iodine value of the cat kidney fat was due to a process of fatty infiltration of the kidney. This view was based on the fact that the cat kidneys contained, on the average, about 5 % of fat compared with the more usual value of 2–3 % for other animals, and that sections of the kidney stained for fat showed an abnormally large number of fat droplets lining the tubules. Assuming that 3 % of the

fats had the customary iodine value for organ fat of 135, which is true for most animals, then the additional 2 % represents infiltrated fat. If the infiltrating fat is adipose tissue fat, and it is to be presumed that Mottram intended this, then the iodine value of the mixed acids would be theoretically 107. This is greatly in excess of the observed iodine value of approximately 60 for the fatty acids from the cat's kidney. The infiltration of adipose tissue fat of iodine value 65 could obviously never account for an iodine value of the mixed acids lower than this value, yet in practice values as low as 50 are observed. It is found experimentally that the iodine value of adipose tissue fatty acids from cats is always about 65. For Mottram's view to be correct, therefore, it must mean that the iodine value of the fatty acids of the "élément constant" for the cat's kidney must be considerably below the accepted normal value of 135. In any case an infiltration hypothesis would not account for the remarkable fluidity of the acids from the cat's kidney. Adipose tissue fatty acids at room temperatures are quite solid, whereas the renal fatty acids of the same or even lower iodine value, are semi-liquid. There must be some fundamental difference in the nature of the fat in the kidney of the cat compared with that of other animals, for it is impossible by making use of values which are true for the latter to account at once for the lower iodine value and the more liquid nature of the fatty acids obtained from the cat's kidney. The presence of some fatty acids which possess a very low or zero iodine value and a high fluidity, for example liquid saturated acids, would reconcile these observations. The experimental procedure was, in consequence, directed towards the isolation of such acids if these were present. The actual isolation of completely saturated fatty acids which are liquid at room temperature confirms the truth of the above suggestion. The very limited amount of these acids at present available makes it impossible to define their constitutions. It is hoped however to proceed with the investigation of this problem when sufficient quantity of the material has been collected; in this preliminary investigation the loss of material has been very heavy, but for the future preparations it is believed that the acids will be isolated in greater yield by a much simpler process.

So far as is known the only naturally occurring liquid saturated fatty acids with more than fourteen carbon atoms are the tuberculostearic and phthioic acids found by Anderson [1929] and Anderson and Chargaff [1930] among the fatty acids from tubercle bacilli. These acids are isomeric with stearic and cerotic acids respectively. Branching of the chain may be a possible explanation of the liquid nature of these acids, since for example α-amyl-n-nonoic acid is still liquid at $-10°$ and β-butyl-n-decoic acid has M.P. 4°.

The experimental evidence suggests that these liquid saturated acids are present in both the simple glyceride and phospholipin fractions of the fats in the cat's kidney. Both these fractions are noticeably more liquid than the corresponding fractions from beef kidney although the iodine values of the latter are much higher. The differences in iodine value are more marked in

the simple glyceride fraction, the values from the beef kidney being almost double those from the cat's kidney. On saponification, beef kidney gives rise to solid acids from both the simple glyceride and phospholipin fractions. The glyceride fraction from cat's kidneys gives rise on saponification to acids which are mainly liquid, whilst the phospholipins yield a waxy product. The inference is that it is in the simple glycerides that the larger proportion of liquid saturated acids is present. The solid saturated acids are apparently present to a greater extent in the phospholipins.

The occurrence of this type of liquid saturated fatty acids in the cat's kidney raises questions of no little physiological interest, but a discussion of these may more appropriately be reserved for a future communication.

Summary.

The percentage amounts and iodine values of the fatty acids from the kidneys of 20 cats have been determined. The mixed fatty acids obtained by saponification of the kidney fats have been examined. They are shown to consist of palmitic, stearic, oleic and linoleic acids, together with at least two new liquid saturated acids. The constitutions of the latter have not yet been ascertained. A comparison has also been made between the simple glyceride and phospholipin fractions obtained by the same processes from cat and beef kidneys.

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