SOME OBSERVATIONS ON THE INTRACELLULAR LIPID IN THE KIDNEY OF THE CAT

By MARY C. LOBBAN

Physiological Laboratory, University of Cambridge

Smith (1920) gave the first detailed account of the occurrence of peculiar intracellular lipoid deposits in the kidney of the normal adult cat. Smith noted that these fatty deposits were confined to the cells of the kidney cortex, and also that the amount of fat present did not vary with the depot fat, but did vary with age, being least in the young kitten and greatest in the old cat. At a later date, Modell (1933) confirmed and enlarged upon Smith's findings. He demonstrated that the intracellular lipoid deposits were confined to 'the convoluted portions of the nephron', and also that it was not the kidney of the very old cat, but that of the pregnant female, which showed the presence of the greatest amount of lipid. No other variation in the amount of lipid present which could be correlated with sexual activity was mentioned by either Smith or Modell, and no further indication was given of any sex differentiation. Also, as all the sections on which these early observations were made were treated with general fat-colorants only, such as Scarlet Red and Sudan III, no specific information can now be gathered as to the type of fat present.

More recently, evidence has been accumulating for the existence of a marked difference between the kidneys of the two sexes in species other than the cat, not only in so far as the actual size and weight of the organ is concerned (Selye, 1939, 1940; Martens & Nylen, 1946) but also with regard to intracellular reactions. Such work includes that of Eschenbrenner & Miller (1945) on sex differences to chloroform-induced kidney necrosis in mice; Kochakian (1944, 1945) on the effects of castration and hormone administration and the distribution of phosphatases in the mouse kidney; and Oster & Oster (1946) on changes in the cell aldehydes of the rat kidney during the oestrous cycle. Modell (1933) had also suggested that it might be worth while to investigate the kidneys of carnivores other than the cat for the presence of intracellular lipids, and in this connexion MacNider (1945) has recently reported on their occurrence in the dog, while Hewer, Matthews & Malkin (1948) have demonstrated the presence of lipids in the kidney cells of the tiger, lion and other large wild cats. Smith, in her original paper on this subject, indicated that varying quantities of lipids could be demonstrated in the kidneys of the dog, rat and rabbit, although the general picture presented by the kidneys of these species was in no case as striking as that found in the kidney of the cat. Gairns & Morrison (1949) have recently reinvestigated the lipoid deposits in the cat's kidney, with results agreeing, in the main, with those already described by the earlier workers.

It would seem that two main points emerge from the work described in the preceding paragraphs. First, that the occurrence of intracellular lipids, in varying amounts, is a perfectly normal finding in the kidney cortex of the adult cat; and, secondly, that in rodents cellular differences may be demonstrated between the kidneys of the two sexes, and in the kidney of the female at different stages of the

oestrous cycle. The present work was undertaken with the object of seeing whether any connexion could be established between these two main observations, and whether the amount of lipid in the cells of the cat's kidney does, in fact, bear any relationship to the sexual state of the animal. Since the kidney bears a close phylogenetic and embryological relationship to the adrenal cortex and to the gonads, both of which produce appreciable amounts of lipids in the form of steroids, it was also thought that part, at least, of the kidney lipids might well be steroid in character. In an attempt to produce evidence in support of this hypothesis, the work was extended to include a histochemical investigation of the nature of the intracellular kidney lipids.

METHODS

Experimental animals. The kidneys of fifty-five adult cats and thirty-two kittens of various ages were examined. The adult cats were killed either by bleeding, under suitable anaesthesia, or by the intra-cardial injection of pentobarbitone sodium. All the kittens used in the investigation were killed with intra-cardial pentobarbitone sodium.

Fixation and staining. Slices of kidney tissue from both kidneys of each animal were fixed for 3 days in Baker's formal-calcium fixative (Baker, 1944). In some cases, paraffin sections were cut at a thickness of 7 μ, and were stained by the Azan and iron-haematoxylin methods. Frozen sections of all the kidneys were cut at approximately 15 μ, and were treated with Sudan III and haematoxylin and with Sudan Black. The latter is to be preferred as a fat-colorant, as it is believed to be more soluble in lipids other than triglycerides than is Sudan III (Baker, 1946).

Histochemical methods. Frozen sections of each kidney were subjected to the Schultze reaction for steroids (adapted from Lison, 1936); the phenyl hydrazine reaction for ketosteroids (Bennett, 1940); and the acid haematin and pyridine extraction tests for the presence of phospholipines (Baker, 1946).

Determination of sexual activity. In all the adult cats, the sexual state of the animals was ascertained by direct histological examination of the gonads.

RESULTS

A. *Presence and distribution of lipids*

In all the adult cats, marked individual differences were noted in the occurrence and distribution of the intracellular kidney lipids, although the two kidneys from any one cat always presented the same appearance. Sudanophilic material was present in the kidneys of all normal, sexually active male cats, but only in moderate amounts (Pl. 1, fig. 1). In sexually inactive male cats, however, the kidney cortex showed very heavy lipoid deposits. In all the male cats which were examined, the kidney showing the greatest amount of intracellular Sudanophilic material was that of the senescent animal, with testes devoid of any signs of spermatogenesis, a female type of fat distribution over the abdominal muscles, and abnormally-developed mammae (Pl. 1, fig. 2). An appreciable increase above normal in the amount of intracellular kidney lipid was also found in the young adult male cat, three weeks after surgical castration (Pl. 1, fig. 3).

The kidneys of the female cats showed a much wider variation in the amount of

intracellular lipid present than did those of the males. No lipid, or only a trace, was found in the kidney of the anoestrous female (Pl. 1, fig. 4), while moderate amounts were demonstrable in the kidneys of pro-oestrous and oestrous animals (Pl. 1, fig. 5). The kidneys of non-pregnant cats in the luteal phase of the oestrous cycle showed fairly heavy lipoid deposits (Pl. 1, fig. 6) while, as stated by Modell in the early work on the subject, the most heavily lipid-laden kidney of all was that of the pregnant cat in the early stages of pregnancy (Pl. 1, fig. 7).

In all the adult cats where intracellular lipids were present, the lipids were typically confined to the convoluted portions of the nephron, with the exception of the junction of the proximal convoluted tubule with the descending limb of Henle's loop. The greater part of the lipid was present in the cells of the proximal convoluted tubules, the distal convoluted tubules being nearly always completely lipid-free, even in a heavily fat-laden kidney (Pl. 1, fig. 8). Very little or no lipid was found in the cells of the kidney cortex of the young kitten (Pl. 2, fig. 9) and the beginning of a typical adult type of fat distribution was rarely found in kittens of less than 2 months of age, and often not until much later, e.g. 4–5 months. These small amounts of intracellular lipids were only found in the kidneys of male kittens, as a general rule, although in one case distinct traces of lipid were found in the kidneys of two, day-old female kittens from the same litter. Lipids were first seen in the form of discrete droplets near the junction of the proximal convoluted tubule with the descending limb of Henle's loop, and it would appear that the lipids extend back towards the glomerulus with increasing age. In older kittens, where traces of lipid were present, the lipoid droplets were more readily coloured with Sudan Black than with Sudan III.

A careful examination of similar sections gave an indication of the presence of two types of Sudanophilic material within the cells of the kidney tubules. One type occurred in the form of relatively large droplets (2–5 μ in diameter) and was coloured both by Sudan III and Sudan Black, while the other, which occurred as much finer droplets, was coloured by Sudan Black only. The two types varied in parallel fashion, except in the kidney of the young male kitten which was just beginning to accumulate lipids, where the type staining with Sudan Black only was often present without the Sudan III staining type. It was also observed that, in the moderately lipid-laden kidney, the lipoid droplets were situated for the most part towards the base of the cells, away from the brush border and the lumen of the tubule. Occasionally, large Sudanophilic globules were seen lying free in the lumen, but these were invariably associated with the presence of damaged cells, and it is thought that they do not occur naturally in this situation, but had most probably been extruded during the processes of freezing and sectioning. Modell (1933) stated that varying quantities of lipids could be demonstrated in the urine of the cat, but all attempts to confirm this observation during the present work have been entirely unsuccessful, even when the urine was obtained from cats which were subsequently shown to possess heavily fat-laden kidneys. If this finding is correct, it would seem that the domestic cat differs markedly from the large wild cats in this respect, as the urine of the tiger has been shown to contain large quantities of lipoid material (Hewer *et al.* 1948).

B. *Histochemical observations*

All three histochemical tests were carried out on formal-calcium fixed material. The frozen sections used for the Schultze and phenyl-hydrazine reactions were thoroughly washed in several changes of distilled water, before being treated with the appropriate reagents. The phenyl-hydrazine and acid haematin and pyridine extraction tests were carried out exactly as described by Bennett (1940) and Baker (1946) respectively, but certain modifications were made in the technique of the Schultze reaction. In this test, it was found that a reagent consisting of 67 % concentrated sulphuric acid and 33 % glacial acetic acid (v/v) was more effective than the original Schultze reagent (Lison, 1936), which consisted of equal parts of the two acids, and also that the rapidity with which the colours developed and the final intensity of the colours were further improved if the reagent was kept for at least 2 days in a loosely-stoppered bottle, before use. All the sections used for the Schultze reaction were first incubated for 24 hr. in a 2·5 % solution of iron alum at 37° C.

In all the kidney sections, the acid haematin and pyridine extraction techniques yielded uniformly negative results, while in the case of young kittens and anoestrous female cats, where the kidney sections contained no Sudanophilic material, the Schultze and phenyl-hydrazine reactions were also negative. In all the kidneys where Sudanophilic material was present, however, the phenyl-hydrazine reaction was definitely positive, and these sections also yielded a colour reaction with the Schultze reagent. In all cases, the positive reactions obtained with these reagents were confined to the kidney cortex, and the extent and intensity of the colours obtained varied according to the amount of Sudanophilic material which had previously been shown to be present. The colour which was produced in the Schultze reaction was not the intense blue-green which is given with this reagent by cholesterol and its esters, the androgens, and certain of the adrenal cortical steroids, but was a definite pink, which developed relatively slowly, and remained stable for periods extending up to 3 hr. This pink colour could be exactly reproduced in the test-tube by adding concentrated sulphuric acid to an equal volume of a chloroform solution of oestrone or oestradiol (1 mg./ml.) to which a trace of cholesterol had been added, and diluting the resulting brilliant yellow, fluorescent solution with three parts by volume of distilled water. The colour so obtained was intensified by the addition of a few drops of a 2·5 % solution of iron alum. It is thought that this pink coloration is closely allied to the red colour given by the naturally occurring oestrogens with the Kober colour reaction (Kober, 1931; Brown, 1952). The fact that a pink colour identical with that observed in the lipid-containing kidney has also been obtained in the follicular fluid and zona granulosa of the Graafian follicles in the Schultze-treated ovary, and in the Sertoli cells of the testis, provides additional support for this view.

The histochemical results, although by no means conclusive, do seem to give some indication that part of the intracellular lipids in the kidney cortex of the cat are possibly steroid in character. If this be so, the steroids present are not likely to be cholesterol alone, the androgens, or the main adrenal cortical steroids, all of which give an intense green or blue-green colour both in Schultze-treated sections and in the test-tube with concentrated sulphuric acid, water and iron alum. There is, how-

ever, a distinct possibility that they may be allied to the naturally occurring oestrogens. Further histochemical investigations are now being undertaken in an attempt to clarify this point.

DISCUSSION

The observations described in this work show that intracellular lipid, in varying amounts, is present in the kidneys of all adult cats, except in the anoestrous female. Moderate amounts of lipids are present in the kidney cortex of the oestrous female and the normal, sexually active male, while a somewhat heavier deposition of lipids is found in the kidneys of the pseudopregnant female and the surgically castrated male. The heaviest lipoid deposits, however, are found in the kidney of the pregnant female and the senescent, sexually inactive male. In every case, the lipids are confined to the cells of the kidney cortex, and there is some indication that they may be, at least in part, of a steroid nature.

In the female cat, it would appear that the amount of intracellular lipids present in the kidney cortex at any one time bears a direct relationship to the sexual state of the animal. The anoestrous female has no lipid (or, occasionally, only a trace) in the kidney cortex, whereas the oestrous female shows an appreciable amount, the lipoid droplets being scattered throughout the epithelium of the proximal convoluted tubules. The amount of lipid present increases throughout oestrus, and reaches a maximum in the luteal phase of the cycle; if the cat becomes pregnant, the lipoid deposits are very heavy indeed, the kidney cortex appearing to be packed with Sudanophilic material. It is presumed that towards the end of pregnancy or pseudopregnancy the amount of lipid present in the kidney tubule cells begins to diminish, and eventually the cat returns to the anoestrous state, with a lipid-free kidney. The diminution in kidney lipid at this stage has not yet been observed, but adult, anoestrous cats with the scars of old corpora lutea in their ovaries (and, in one case, a cat which was known to have given birth to a litter of four kittens some 15 weeks previously) have been found to possess kidneys which are just as free of lipids as are those of the young female which has not yet reached sexual maturity. It would seem, then, that the laying down of lipids in the kidney of the female cat occurs only at times when there are greatly increased amounts of the female sex hormones in the circulation. If it is believed that these female hormones are the cause of lipid accumulation in the kidney, then progesterone has a more marked effect in this respect than have the oestrogens, since the deposition is considerably greater in the luteal phase of the cycle than in the follicular phase.

The picture presented by the lipids in the kidneys of male cats is somewhat more difficult to interpret. The first apparent anomaly arises in the normal, sexually active male, where moderate amounts of intracellular kidney lipids are invariably found. It is well known that small amounts of the female sex hormones are always found in the circulation of the male, and at first sight it might appear permissible to suggest that the kidney lipid content in the male is maintained under the influence of such hormones. When, however, the male kidney is compared with that of the anoestrous female (which animal, even though sexually quiescent, is surely to be regarded as more female, both in general characteristics and in steroid balance, than is the normal male) which is conspicuously free from intracellular lipids, it does

not seem that such an explanation can hold good. It is possible, however, that the hormones responsible may be the anterior pituitary gonadotrophins. It is now generally accepted that the luteinizing hormone (L.H.) of the female is identical with the interstitial cell stimulating hormone (I.C.S.H.) of the male, and it can readily be seen that there may be periods when there is considerably more of this principle in the blood of the normal male than in that of the female, i.e. when the female is in the anoestrous state. Such an explanation could be satisfactorily applied both to the maintenance of the intracellular kidney lipids in the male, and to the laying down of lipids which occurs during sexual activity in the female. The pituitary gonadotrophins could also be the stimulus which causes the laying down of abnormally large amounts of lipid in the kidney of the surgically castrated male; this explanation would be well in accord with the increase in the number of β cells which is regularly observed in the anterior pituitary after castration.

The increase in the amount of kidney lipids observed in the senescent male cat cannot, however, be ascribed to stimulation by the pituitary gonadotrophins. Indeed, sensecence is to be regarded as being directly due to a failure in pituitary gonadotrophin production, histological examination of the testes of senescent animals showing complete absence of spermatogenesis together with the presence of shrunken, inactive interstitial cells, the latter appearing to be conspicuously poor in steroid content. The Sertoli cells in such a testis, however, are remarkable for their very large size and greatly increased lipoid content. The lipoid droplets in these cells have been found to yield a pink colour with the Schultze reagent which is identical with that already described in the cells of the lipid-containing kidney, and it is suggested that, under these conditions, the Sertoli cells may well be the source of appreciable quantities of oestrogens. This view is supported by the work of Huggins & Moulder (1945) who have correlated the occurrence of Sertoli cell tumours with feminism in the male dog; the picture presented by such dogs, with their tendency to lay down increased amounts of subcutaneous fat, loss of sexual function, attractiveness to other males, and abnormally developed mammae, greatly resembles the condition observed in the senescent male cat. No data is available on the condition of the dog's kidney under these circumstances. In the senescent cat, however, it is suggested that the very heavy lipoid deposits which are observed in the cells of the kidney cortex may be due to increased amounts of female steroids in the circulation which, in turn, may be produced by the hyperactive Sertoli cells. There is, however, the possibility that if the kidney steroids are indeed of the oestrogen type, the reverse argument may be true, in that it is the kidney oestrogens which are producing the changes in the testis, secondary sexual characteristics and in the adrenal cortex (Lobban, 1952). If this be so, no explanation can, as yet, be offered for the increased laying down of lipids in the kidney of the senescent male cat. It is clear that further work must be carried out on the hormone balance in the male cat before this problem can be solved: such work is now in progress.

SUMMARY

1. A study has been made of the occurrence and distribution of intracellular lipid in the kidneys of fifty-five adult cats and thirty-two kittens.

2. It has been found that a marked increase in the laying down of intracellular kidney lipids is associated with the loss of sexual function in the male cat and with the luteal phase of the oestrous cycle in the female.

3. Histochemical evidence has been put forward which suggests that part, at least, of the intracellular lipid is of a steroid nature, and that the steroids present are more likely to be allied to the oestrogens than to the male sex hormones.

4. The significance of these findings and the possible role of the anterior pituitary gonadotrophins in connexion with them has been discussed.

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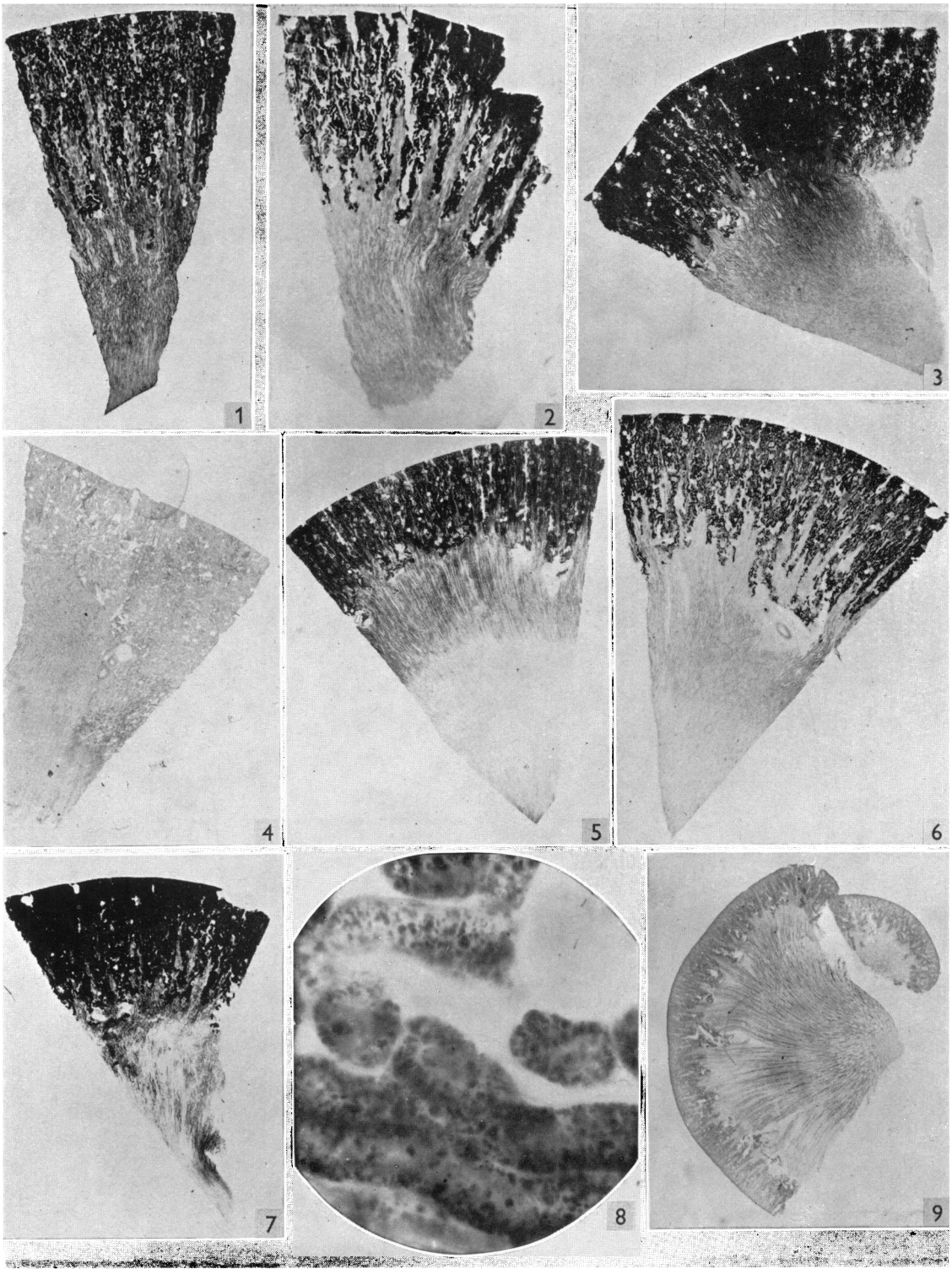
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EXPLANATION OF PLATE

Figs. 1–7. Frozen sections of kidneys of adult cats, Sudan Black, × 7. Fig. 1. Normal male. Fig. 2. Senescent male. Fig. 3. Male, three weeks after surgical castration. Fig. 4. Anoestrous female. Fig. 5. Oestrous female. Fig. 6. Non-pregnant female in the luteal phase of the oestrous cycle. Fig. 7. Pregnant female, in third week of pregnancy.

Fig. 8. High-power photomicrograph of kidney of normal male cat, showing lipoid droplets in the epithelium of the proximal convoluted tubules. Sudan Black, × 175.

Fig. 9. Frozen section of kidney of 6-week old kitten, Sudan Black, × 7.