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STRUCTURAL VARIATIONS IN THE ADRENAL CORTEX OF THE ADULT CAT

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The existence of a functional inter-relationship between the adrenal cortex and the gonads is well recognized in a number of species, and the subject has recently been comprehensively reviewed (Parkes, 1945). In some rodents, actual visible changes with sexual activity, both in intracellular lipid content, and in histological structure, have also been demonstrated in this gland. The intracellular lipid in the adrenal cortex of the rat has been shown to undergo changes in amount and distribution which occur regularly at certain stages of the reproductive cycle (Korenchevsky & Hall, 1938). The innermost zone of the adrenal cortex—called by some workers the 'x-zone' or 'variable zone'—has been shown to undergo striking degenerative changes in the mouse, associated with puberty in the male, and pregnancy in the female (Masui & Tamura, 1926; Miller, 1928; Deanesly, 1928). All the evidence suggests that there is a close relationship between the observed changes and variations in sexual activity in the animals which have been studied.

A sex difference in the staining reactions of the adrenal cortex of the male and female cat has already been recorded (Bennett, 1940), while Davies (1937) has described a transitory inner zone in the adrenal cortex of the foetal cat. The present work arose from certain observations which were made during the course of a histological study of some of the derivatives of the coelomic epithelium in the cat. It was noticed that great variations occurred in the structure of the adrenal cortex in the adult animal, and that these variations could not be ascribed to a simple sex difference. These variations related primarily to the degree of development of the innermost zone of the gland, the zona reticularis. As this zone occupied the same position in the adrenal cortex, and showed similar staining reactions to those of Deanesly's 'variable zone' in

the adrenal cortex of the mouse, it was decided to investigate the structure of the adrenal cortex in a number of adult cats of both sexes, with special reference to the sexual state of the animals at the time of the investigation. It was hoped that such a study might reveal differences in the structure of the adrenal cortex which could be related to the sexual activity of the animal.

METHODS

Experimental animals. A histological examination was made of the adrenal glands of fifty-five adult, healthy cats. The animals were killed either by bleeding, under suitable anaesthesia, or by the intracardial injection of pentobarbitone sodium.

Fixation and staining. Both adrenal glands were removed from each animal immediately after death, and were fixed for 3 days in Baker's formal-calcium fixative (Baker, 1946). For the investigation of cell structure, paraffin sections were cut at a thickness of 7μ , and were taken longitudinally through the midline of each gland. Since the zone which was of particular interest in this work, the zona reticularis, showed that affinity for azo-carmines and other acidic dyes which is commonly termed acidophilia, Azan staining was used as a routine method throughout the investigation. A few frozen sections were treated with Sudan black to demonstrate the presence of lipids, but as some of the cats from which the adrenal glands were taken had previously been subjected to procedures which might have caused some stress, no systematic study of the lipid content of the glands was carried out.

Determination of the F/R ratio. The relative proportions of the zona glomerulosa, zona fasciculata and zona reticularis in each adrenal cortex were determined in its thickest and thinnest portions by the use of a micrometer eye-piece. The junction between the zona fasciculata and zona reticularis was very sharply defined, as the cells of the latter zone showed an intense acidophilia, while those of the former possessed pale-staining cytoplasm. The acidophilic cells showed the comparatively small size, darkly-staining nuclei and reticular arrangement commonly recognized as typical of the zona reticularis, so, in every case, the junction between acidophilic and non-acidophilic tissue was taken as the dividing line between the two zones. The widths of the zones were expressed as the number of divisions of the eye-piece scale which they were seen to occupy under standard conditions of lighting and magnification. From these estimates the fasciculata/reticularis (F/R) ratio—simply the ratio of the width of the zona fasciculata to that of the zona reticularis—was deduced for each gland, and provided a convenient measure for comparison of the development of the latter zone in glands from different animals.

Note. The degree of shrinkage undergone by the adrenal glands during the processes of fixation, dehydration and embedding was not determined for all the specimens. In the later part of the work, however (from cat 24 onwards) measurements were taken, and the shrinkage was found to be of the order of 15%, this figure appearing to be independent of the amount of lipid present in the glands.

Determination of sexual activity. In all cases, the sexual state of the cats used during the work was ascertained by histological examination of the gonads. In the later stages of the work, the adrenal glands of cats in which the sexual condition had been influenced by experimental means (e.g. by castration, stilboestrol implantations, and castration with subsequent injections of testosterone propionate) were also studied. In the male cats of this series, unilateral adrenalectomy was performed before the state of sexual activity was interfered with, so that a direct comparison of the adrenal cortices before and after the experimental procedure was made possible. Unilateral adrenalectomy was not, as a rule, carried out in the case of the female cats, as it was thought that the comparatively severe operative interference involved might well lessen the chances of inducing ovarian activity.

RESULTS

Normal cats

In all cats, the width of the zona glomerulosa was remarkably constant, and the width of the zona fasciculata also showed very little variation. The width of the zona reticularis, however, varied considerably in different individuals, although the F/R ratio was always the same in the right and left glands from any one cat. The F/R ratios could be divided into four groups, the individual animals within each group showing certain characteristics in common with regard to their state of sexual activity (Tables 1-4).

TABLE 1. Fasciculata/reticularis ratios in normal male cats

Cat	Description	Widths of zones			F/R ratio
		Z.G.	Z.F.	Z.R.	
1	Young adult	3	30	12	2.50/1
2	Adult	3	32	13	2.46/1
3	Adult	3	31	12	2.58/1
7	Adult	3	29	11	2.64/1
9	Adult	3	32	12	2.67/1
13	Adult	3	30	13	2.31/1
24	Adult	3	28	10	2.80/1
25	Adult	3	30	11	2.73/1
33	Adult	3	18	6	3.00/1
41	Adult	3	31	12	2.58/1
49	Adult	3	30	11	2.73/1
50	Adult	3	28	9	3.11/1
51	Adult	3	31	11	2.82/1
52	Adult	3	30	10	3.00/1
53	Adult	3	29	10	2.90/1

Mean F/R ratio—2.72/1 (approx. 3/1).

Widths of zones expressed in numbers of divisions of micrometer eye-piece scale. Z.G. zona glomerulosa, Z.F. zona fasciculata, Z.R. zona reticularis.

TABLE 2. Fasciculata/reticularis ratios in sexually inactive male cats

Cat	Description	Widths of zones			F/R ratio
		Z.G.	Z.F.	Z.R.	
4	Castrate	3	32	30	1.07/1
8	Senescent	3	31	25	1.24/1
12	Castrate	3	33	33	1.00/1
14	Castrate	3	30	22	1.36/1
19	Castrate	3	24	24	1.00/1
20	Immature	3	18	18	1.00/1
22	Immature	3	27	27	1.00/1
38	Immature	3	19	18	1.06/1
40	Senescent	3	30	30	1.00/1
42	Castrate	3	32	32	1.00/1
54	Castrate	3	30	29	1.03/1

Mean F/R ratio—1.07/1 (approx. 1/1).

Widths of zones expressed in numbers of divisions of micrometer eye-piece scale. Z.G. zona glomerulosa; Z.F. zona fasciculata; Z.R. zona reticularis.

TABLE 3. Fasciculata/reticularis ratios in sexually quiescent female cats

Cat	Description	Widths of zones			F/R ratio
		z.G.	z.F.	z.R.	
5	Anoestrous adult	3	35	12	2.92/1
11	Immature	3	35	3	11.67/1
17	Immature	3	32	9	3.56/1
18	Immature	3	30	7	4.67/1
21	Anoestrous adult	3	25	12	2.08/1
26	Anoestrous adult	3	30	18	1.67/1
27	Anoestrous adult	3	24	12	2.00/1
29	Anoestrous adult	3	30	12	2.50/1
32	Anoestrous adult	3	31	12	2.58/1
34	Immature	3	24	12	2.00/1
35	Anoestrous adult	3	27	17	1.59/1
39	Immature	3	28	18	1.56/1
43	Anoestrous adult	3	30	18	1.67/1
44	Anoestrous adult	3	29	12	2.42/1
46	Anoestrous adult	3	31	16	1.94/1

Widths of zones expressed in numbers of divisions of micrometer eye-piece scale. z.G. zona glomerulosa; z.F. zona fasciculata; z.R. zona reticularis.

TABLE 4. Fasciculata/reticularis ratios of sexually active female cats

Cat	Description	Widths of zones			F/R ratio
		z.G.	z.F.	z.R.	
30	Pro-oestrous	3	30	21	1.43/1
31	Pro-oestrous	3	30	21	1.43/1
45	Oestrous	3	30	27	1.11/1
6	Early pseudo-pregnant	3	33	27	1.22/1
47	Early pseudo-pregnant	3	29	26	1.12/1
28	Pseudo-pregnant	3	30	30	1.00/1
48	Pseudo-pregnant	3	27	26	1.04/1
36	Pseudo-pregnant	3	33	33	1.00/1
16	Late pseudo-pregnant	3	21	18	1.17/1
37	Late pseudo-pregnant	3	24	18	1.33/1
10	Pregnant	3	28	29	0.97/1
15	Pregnant	3	28	30	0.93/1
23	Pregnant	3	27	33	0.82/1
55	Pregnant	3	28	30	0.93/1

Widths of zones expressed in numbers of divisions of micrometer eye-piece scale. z.G. zona glomerulosa; z.F. zona fasciculata; z.R. zona reticularis.

Group 1, of normal male cats, with functional testes, showed an F/R ratio of approximately 3/1 (Fig. 1a).

Group 2, of sexually inactive males, showed an F/R ratio of 1/1. The ratio in this second group was the same whether the lack of sexual activity was due to immaturity, old age, or to surgical castration (Fig. 1b-d).

Group 3, of sexually quiescent female cats, showed F/R ratios covering a wide range of from 11/1 to 2/1, the ratio tending to approach the lower value as the animals became more mature (Fig. 2a, b).

Group 4, consisting of pro-oestrous, oestrous, pregnant and pseudo-pregnant cats, showed an F/R ratio of approximately 1/1 (Fig. 2c-f). In this last group, the development of the zona reticularis was most marked in the adrenal glands

from pregnant cats, where the width of the zona reticularis sometimes even slightly exceeded that of the zona fasciculata, and the acidophilia of the reticularis was very intense.

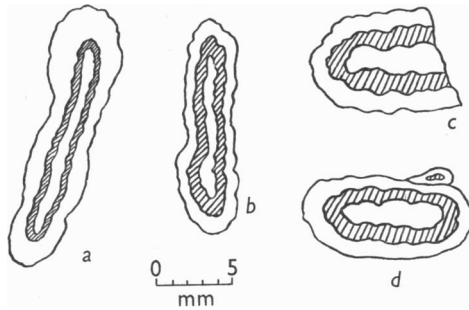


Fig. 1. Line drawings to show the extent of the zona reticularis in adrenal glands of male cats. (a) normal, (b) immature; (c) senescent, (d) castrated. Reticularis indicated by shading.

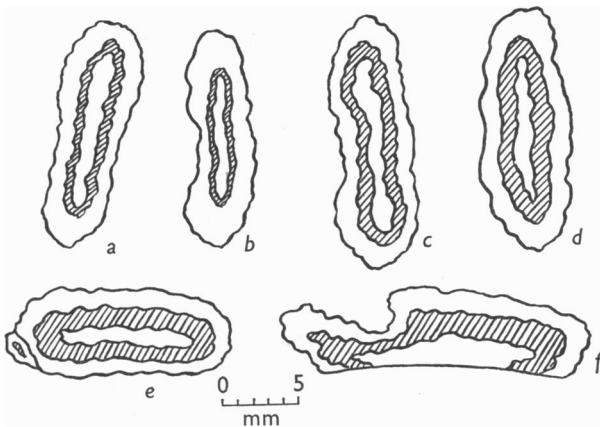


Fig. 2. Line drawings to show the extent of the zona reticularis in adrenal glands of female cats. (a) anoestrous adult, (b) immature, (c) pro-oestrous, (d) oestrous, (e) pseudo-pregnant, (f) pregnant. Reticularis indicated by shading.

Cats in which the state of sexual activity was experimentally altered

In this group, three preliminary experiments were carried out in which young adult cats were subjected to unilateral adrenalectomy alone, to investigate the possible effects of compensatory hypertrophy of the remaining adrenal gland upon the interpretation of results. Two young adult male cats and one young adult, anoestrous female cat were used, and it was found that although the animals remained in perfectly normal condition, and showed no signs of adrenal cortical insufficiency, no appreciable enlargement of the remaining adrenal gland had occurred 4 weeks after the operation. The slight enlarge-

ment which did take place (in one young male cat) was distributed equally throughout the adrenal cortex, so that the F/R ratio for that cat was unaltered.

Male cats. Three young adult male cats showed on unilateral adrenalectomy an F/R ratio of 3/1, typical of the normal, sexually active male. They were then castrated (the testes, when examined histologically, showed definite signs of active spermatogenesis) and kept for 4 weeks, after which time the animals were killed and the remaining adrenal glands were examined. In all three cases, histological examination revealed an F/R ratio of approximately 1/1 in the post-castration adrenal gland (Fig. 3*a, b*). Two other young adult male cats were subjected to the same procedure, and were, in addition, given daily injections of testosterone propionate (1 mg per diem, intramuscularly) during the post-operative period. Examination of the remaining adrenal gland after four weeks of this treatment revealed that the original F/R ratio of 3/1 had been maintained in both cats, in spite of the absence of testicular tissue (Fig. 3*c, d*).

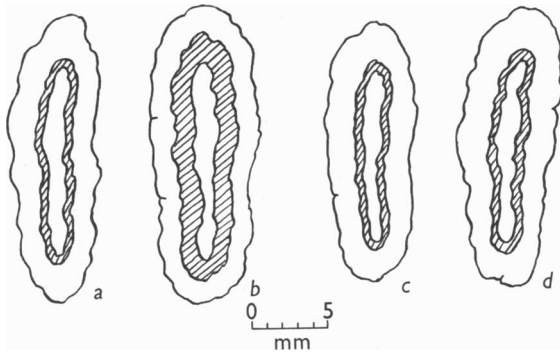


Fig. 3. Line drawings to show the effect of castration and castration with injections of testosterone propionate on the extent of the zona reticularis in the male cat. (*a*) adrenal gland removed at time of castration; (*b*) remaining adrenal gland, 4 weeks after castration; (*c*) adrenal removed at time of castration; (*d*) remaining adrenal gland, after 4 weeks of testosterone propionate injections. Reticularis indicated by shading.

Female cats. Stilboestrol dipropionate tablets (25 mg) were implanted into the subcutaneous tissue of the flank of two young, sexually quiescent adult female cats, and the animals were killed on the fourth and fifth day after implantation, some 6 hr after they had first showed signs of oestrus-like behaviour. At death, both cats were found to have mature follicles in their ovaries (Fig 4*a*). In both cases, the F/R ratio was approximately 1/1 (Fig. 4*b*). Three more female cats were brought into oestrus in the same way, and were kept until the signs of oestrus had abated, the animals being frequently stroked and handled during this period, in an effort to induce ovulation. The induced oestrous period commenced on the fourth day after implantation in one cat, and lasted until the ninth day, while the other two cats showed oestrus-like

behaviour from the second to the fifth day and the third to the fifth day. The animals were all killed 24 hr after the cessation of oestrus. In all three cases fresh corpora lutea were found in one (two cats) or both (one cat) ovaries, and the F/R ratio of the adrenal cortex was 1/1 (Fig. 4*c, d*). The observations upon

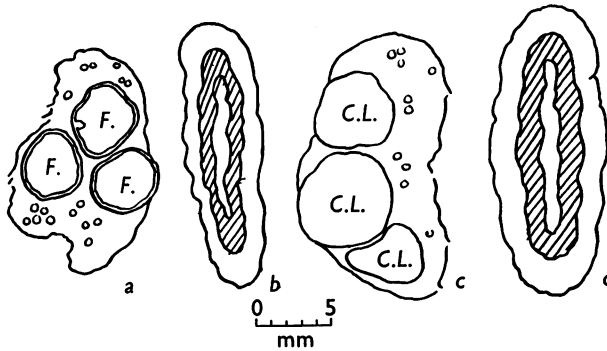


Fig. 4. Line drawings to show the effect of stilboestrol implants on the ovaries and zona reticularis of the female cat. (*a*) ovary, 4 days after stilboestrol implantation; (*b*) adrenal gland, 4 days after implantation; (*c*) ovary, 24 hr after cessation of induced oestrus; (*d*) adrenal gland, 24 hr after cessation of induced oestrus. *F*, follicle; *C.L.*, corpus luteum. Reticularis indicated by shading.

the adrenal cortices of these ten cats in which the state of sexual activity had been experimentally altered were thus well in accord with those observations recorded of adrenal glands in normal cats.

DISCUSSION

From the observations recorded in this paper it may be concluded that in the cat the innermost zone of the adrenal cortex, the zona reticularis, is present throughout adult life in both sexes, but that it undergoes structural changes which bear a definite relationship to the activity of the gonads. These changes may be summarized as follows: in the young, sexually immature cat, the zona reticularis shows a much greater degree of development in the male than in the female. Shortly before puberty, the zona reticularis of the young male cat equals the zona fasciculata in width, while in the female of the same age the reticularis is still comparatively narrow. At puberty, however, degenerative changes take place in this zone, and when sexual function is fully established, the zona reticularis in the male cat is found to have decreased to approximately half the width reached just before puberty. The reticularis of the young female cat, however, continues to increase in extent, until it reaches a steady state of development, where it is about as wide as, or slightly wider than, in the sexually active male. In the male cat it then undergoes little further structural change unless the cat be surgically castrated, or becomes senescent, when in

both cases the zona reticularis increases very markedly in extent. In the female cat, however, regular cyclical changes take place, which bear a definite relationship to the phases of the reproductive cycle. These consist of a marked increase in the extent of the zona reticularis during pro-oestrus and oestrus, which is continued over into the post-ovulation period of pregnancy or pseudo-pregnancy, the peak of development being reached during the luteal phase of the cycle. The adrenal cortex of the pregnant or pseudo-pregnant cat thus comes to resemble very closely that of the castrate male. This stage of development in the female is thought to be due to the presence of active luteal tissue in the ovaries, the zona reticularis decreasing in extent and returning to its anoestrous condition as the activity of the luteal cells dies down, since some of the female cats showing a typical anoestrous F/R ratio of 3/1 or 2/1 had the remnants of old corpora lutea in their ovaries. An increase in the width of the zona reticularis may be produced in the male cat by castration, and in the female cat by the 'artificial' induction of oestrus and pseudo-pregnancy. The widening of the zona reticularis brought about by castration in the male cat may be prevented by the administration of testosterone propionate.

The zona reticularis occupies the same position in the adrenal cortex of the cat as does the 'x-zone' in the adrenal cortex of the mouse. The zones also show identical staining reactions in the two species, and both undergo structural changes which are associated with variations in the activity of the gonads. It would seem at first sight, then, that there are strong reasons for supposing that the 'x-zone' and the reticularis are identical. The behaviour of the zone, however, is not identical in the two species. According to Deanesly (1928), the variable zone in the adrenal cortex of the mouse develops more rapidly in the female than in the male. At puberty, the zone undergoes rapid and complete degeneration in the male mouse, while persisting in the female. If the female becomes pregnant, the zone degenerates completely during the first half of pregnancy; otherwise it persists throughout the animal's active sexual life, degenerating very slightly at each successive oestrous cycle. Thus a phase of sexual activity which leads to the complete disappearance of the variable zone in the female mouse is, in the cat, associated with the increased development of this zone.

It is apparent that, in the female cat, the peak of development of the zona reticularis is associated with the peak in the production of the female hormones. The most potent hormone in this respect is progesterone, the secretion of the active corpus luteum. In the male cat, however, the greatest degree of development of this zone is seen when all normal sexual activity is absent. It is suggested that in both sexes, the increased development of this zone of the adrenal cortex is due to what might be termed the ascendancy of the female hormones in the organism as a whole. This state of hormone balance is clear-

cut in the case of the female cat, but is somewhat more complicated in the male. It is true, however, that the point in common between the immature male and the senescent male is the absence of spermatogenesis, with a consequent preponderance of Sertoli cells in the seminiferous tubules of the testis. There is fairly good clinical evidence that in the dog it is the Sertoli cells which are the source of those female steroids which are always present in the male (Huggins & Moulder, 1945), and it is thought that this may also be true for the cat. If this is so, then it is possible that an over-activity of the Sertoli cells could produce a picture in the adrenal cortex of the male cat resembling that found in the adrenal cortex of the pregnant female. This state of affairs obviously cannot hold good for the castrate male. There is, however, some evidence that the kidney, which bears a close phylogenetic relationship to both the adrenal cortex and certain cells in the gonads, may produce appreciable quantities of female-type steroids in the cat, and these steroids may be responsible for the increased development of the zona reticularis. This hypothesis, and the histochemical evidence for it, will be discussed in a later paper.

It has been stated by Hill (1930) that the adrenal gland of the cat does not show any signs of a zona reticularis during post-natal life. This statement is refuted by Davies (1937), who has described the development of an adult reticularis from the part of the fasciculata nearest the medulla. In the present work, a well-marked zona reticularis has been found to be present in every adrenal gland examined. The relationship between the transitory zone in the adrenal cortex of the mouse and the zona reticularis in the adult cat is, however, by no means clear. If the zones in the two species are indeed analogous, then the differences in behaviour which have been observed between them cannot be explained otherwise than on the grounds of a species difference. It has been shown by Davies, however, that the adrenal gland of the cat possesses a large, reticularis-like structure in foetal life, which disappears completely before birth. A similar zone has been demonstrated in the adrenal glands of the larger Felidae by Hill (1937), and both in them and in the domestic cat, the changes observed in this region of the gland closely resemble those which are well recognized in the adrenal glands of primates. Since the transitory zone in the mouse adrenal also eventually undergoes degeneration and disappearance (albeit in post-natal life), it is probable that it corresponds to the 'foetal cortex' in the Felidae and the primates, rather than to the true reticularis as seen in the adult cat.

The simplest interpretation to put upon the observations reported in this paper is that the zona reticularis in the adult cat acts as a secondary sex organ, responding to the changes in the sexual state of the animal in much the same way as, for example, the epithelium of the seminal vesicles. The evidence so far available, however, is not sufficient to say whether this is so.

SUMMARY

1. A histological examination has been made of the adrenal glands of fifty-five adult cats of both sexes.

2. Structural variations have been observed in the zona reticularis of the adrenal cortex which can be correlated with the sexual state of the animal.

3. The significance of the observations has been discussed. It is suggested that the zona reticularis in the cat may react as a secondary sex organ.

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