AGE AND SEX VARIATIONS IN THE FAT OF THE ADRENAL CORTEX OF THE WHITE RAT

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The general distribution of fat in the adrenal cortex of the rat is described by Jackson (1919) and Watson (1927), but no detailed study seems to have been reported. The present paper is a parallel to the systematic studies of Whitehead on the mouse (1933*a*, 1934*a*), guinea-pig (1934*b*, 1935*a*) and rabbit (1936). The results form a basis for experimental work on cortical fat and also bear on the problem of cortical structure.

MATERIAL AND METHODS

The adrenals of 120 normal albino rats of the Wistar strain were studied. The rats were killed so as to form six age groups each consisting of ten males and ten females. The body weights just before death are shown in Table 1. The dates of birth of all rats not over 169 days old were known; none of these rats had been used for breeding. The rats forming the group 'over 169 days' old were probably over a year old; these had been used for breeding and were killed when considered too old to be used again

Table 1. *Body weights (g.) of normal rats*

Age days	Males		Females	
	Mean	Range	Mean	Range
<1	5·7	5·0–6·4	5·0	4·4–5·5
14	23·0	21·0–24·0	22·6	21·0–25·0
29	64·6	58·0–77·0	64·3	53·0–71·0
84	222·0	186·0–274·0	163·8	146·0–180·0
169	288·4	234·0–340·0	189·1	168·0–226·0
>169	293·0	242·0–340·0	225·8	217·0–238·0

Each age group consisted of ten males and ten females: total 120.

for this purpose. The diet consisted of Purina fox chow meal and water. Vaginal smears were made on the thirty sexually mature females for 4 days before death, the technique of smearing and method of interpretation being those of Long & Evans (1922). Four rats were killed in pro-oestrus, seven in oestrus, three in metoestrus and fourteen in dioestrus; the phase of the other two was uncertain. Four rats were killed by a blow on the head, all the others by decapitation.

Both adrenals of each rat were fixed for at least 2 days in 10 % formol saline, prepared by dissolving 8·5 g. sodium chloride in 100 c.c. commercial formalin and diluting the solution to 1 l. with distilled water. Transverse sections cut at 25 μ on a freezing microtome were (*a*) stained for fat with Sudan IV without counterstain, and (*b*) treated by the Schultz method for cholesterol (Kay & Whitehead, 1937).

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RESULTS

The appearance of cortical fat was closely similar in both adrenals of any rat. The range of variation was the same in both sexes, but there were sex differences in the frequency of certain appearances. There was no evidence of any relation between fat and the oestrous cycle. The Schultz test showed that all the fat in all the adrenals contained cholesterol, the distribution of cholesterol being the same as that of fat.

According to its fat content the cortex was divisible into four concentric zones (Pl. 1, fig. 1): (1) a narrow subcapsular zone which was always fat-laden, (2) a narrow zone in which fat was rarely obvious, (3) a broad zone nearly always fat-laden, and (4) a broad juxta-medullary zone with a widely variable fat content. Fat-containing cortical cells were also seen in the medulla of all the adrenals of the newborn rats (Pl. 1, fig. 2) and of one or both adrenals of some rats of each sex at later ages.

The only important variations were in zones 3 and 4. Zone 3 showed regular age variations in size; since the size of zone 3 varied inversely as the size of zone 4 it is unnecessary to give results for both zones, and it will be more convenient to give them for zone 4. Zone 3 showed no obvious regular variation in fat content, but there was one conspicuous irregular variation, namely, the almost complete absence of fat from zone 3 in both adrenals of each of the following rats: at 14 days, a 24 g. male and a 22 g. female; at 29 days, a 61 g. male; at 84 days, a 156 g. female. Zone 4 showed regular age variations in fat content as well as size, and these will now be described in detail.

Table 2. *Numbers of rats showing various sizes of zone 4 at various ages (days)*

Breadth of zone 4 / Breadth of cortex	Males						Females					
	<1	14	29	84	169	>169	<1	14	29	84	169	>169
2/3*	—	6	1	—	—	—	—	7	—	—	—	—
1/2	—	4	7	6	2	1	—	3	—	3	—	—
1/3	—	—	2	3	8	7	—	—	9	7	2	4
1/4	—	—	—	—	—	2	—	—	1	—	6	—
—†	10	—	—	1	—	—	10	—	—	—	2	6

* The fractions in this column are rough estimates obtained by simple inspection; lack of a sharp boundary between zones 3 and 4 precluded the use of exact methods of measurement.

† Zone 4 indistinguishable from zone 3.

The variations in the size of zone 4 are shown in Table 2. It shows that (*a*) in the newborn rats zone 4 was indistinguishable (Pl. 1, fig. 2), the abundant fat of zone 3 extending to the inner border of the cortex, (*b*) at 14 days zone 4 was at its broadest (Pl. 1, fig. 3), (*c*) after 14 days zone 4 became progressively narrower, (*d*) after 14 days zone 4 was on the average narrower in the females than in the males, (*e*) in the females zone 4 continued to narrow beyond 169 days and in the oldest group was usually indistinguishable, as in the newborn rats.

The variations in the fat content of zone 4 are shown in Table 3. It shows that (*a*) at 14 and 29 days fat was absent or virtually absent, (*b*) after 29 days fat was usually scanty (Pl. 1, fig. 4).

Fat in zone 4 was often least scanty in the cortical cells immediately outside the medulla. This phenomenon, henceforward referred to as the 'juxta-medullary fat concentration', was obvious in the following rats: at 84 days, three males (both adrenals of each) and six females (both adrenals of two, left only of three, right only of one);

at 169 days, one male (both adrenals) and six females (both adrenals of five, right only of one); after 169 days, three males (both adrenals of two, left only of one). The juxta-medullary fat concentration is not shown in any of our figures but is illustrated by Jackson (1919, Figs. 3–5) and Andersen & Kennedy (1932, Fig. 2, photographs 3 and 4).

Table 3. *Numbers of rats showing various amounts of fat in zone 4 at various ages (days)*

	Males						Females					
Fat in zone 4	<1	14	29	84	169	>169	<1	14	29	84	169	>169
Absent or virtually so	—	10	10	—	3	—	—	10	10	—	—	—
Scanty	—	—	—	9	7	10	—	—	—	10	6	4
Abundant*	10	—	—	1	—	—	10	—	—	—	4	6

* In inner part of cortex; zone 4 was indistinguishable from zone 3 except in two of the females aged 169 days.

DISCUSSION

The most striking age variation in cortical fat was the decrease in the amount of fat in the inner part of the cortex during the first 2 weeks of postnatal life and the gradual increase of fat in this region, a process appreciable at the end of the fourth week. The fat-free or virtually fat-free zone consisting of the inner half or more of the cortex 14 days after birth, which we have termed zone 4, is the region termed the juvenile cortex by Howard (1938). She found that the juvenile cortex resembled the mouse *X* zone in its time of appearance and in certain cytological respects, but that it differed from *X* zone in the absence of any sex difference in its duration or maximal size and also in the absence of degenerative changes; the juvenile cortex assumed the character of adult cortex by changes in the appearance and arrangement of its cells. Our observations show that the juvenile cortex of the rat resembles non-involuting mouse *X* zone in showing no obvious fat.

The juxta-medullary fat concentrations at first sight suggest cortical involution by fatty change of the type seen in the mouse *X* zone, but the invariable occurrence of the Schultz cholesterol reaction in such concentrations is a point against this suggestion; on the few occasions when the Schultz test has been applied to involution fat (Whitehead, 1934*a* (mouse *X* zone); 1935*b* (rabbit permanent cortex)) the result has been negative, although positive in the normal fat in the same adrenal. Since both normal and involution fat are sudanophil, the Schultz test is useful for distinguishing between them.

Our failure to find any relation between the appearance of cortical fat and the oestrous cycle conflicts with the results of Andersen & Kennedy (1932). According to these workers the characteristics of the oestrous fat pattern (re-expressed in our terminology) are (*a*) absence of zone 2, and (*b*) increased fat in zone 4 including the juxta-medullary fat concentration. Their Fig. 2, photograph 4, shows an adrenal from a rat in oestrus. Zone 2 is absent (we noted absence of zone 2 only once—from the left adrenal of one of the two rats whose oestrous phase was uncertain). Zone 4 contains more fat than in the dioestrous control (their Fig. 2, photograph 3), but the amount present seems well within the normal range for rats in dioestrus. As for the juxta-medullary fat concentration, differences in this exceeding that illustrated are common between the two adrenals of the same rat.

The variations in cortical fat in the rat may be compared with those previously observed in the mouse, guinea-pig and rabbit. The four species were alike in the following respects: (*a*) cortical fat was most variable in the inner part of the cortex, (*b*) fat in older animals was more abundant in the females than in the males, (*c*) the appearance of fat was closely similar in both adrenals of any individual, and (*d*) all normal fat always contained cholesterol. In other respects there were considerable species differences. The mouse and guinea-pig showed three fat zones: (1) a narrow subcapsular zone containing little or no fat, (2) a broad fat-laden zone, and (3) a broad fat-free or virtually fat-free zone surrounding the medulla. The rabbit showed only zones 1 and 2; fat in zone 1 was more abundant than in the mouse or guinea-pig, and zone 2 occupied all the remaining cortex at all ages. The rat was unique in two respects: (*a*) fat in zone 1 was far more abundant than in the other species, and (*b*) the narrow zone 2 of the rat was absent from the other species. Owing to the presence of the rat zone 2, zone 3 in the rat corresponds to zone 2 in the other species and zone 4 in the rat to zone 3 in the mouse and guinea-pig.

The four species also differed in the nature of the age variations. In the mouse fat usually occupied all the permanent cortex while the *X* zone was present; when the *X* zone had disappeared the amount of fat fell but soon showed no progressive change. In the guinea-pig there was a progressive decrease in the amount of fat from 14 days onwards. In the rabbit fat was relatively less abundant after the first month but there was no obvious progressive change. In the rat an early decrease in the amount of fat was followed by a progressive increase.

The results for the four species have a direct bearing on the conduct of experimental work on cortical fat. They provide standards for checking the normality of control material and make it possible to choose particular fat distributions for special study. The wide normal range of variation in the appearance of cortical fat within individual species makes it difficult to establish the occurrence of experimental changes; such a change might consist merely in a shift within the normal range, and it would then be impossible to say whether the fat in any particular adrenal had undergone a change. The close similarity in the appearance of cortical fat in the two adrenals of the same individual suggests that comparison of the two adrenals, one removed before experiment as a control, the other after experiment, would be the best method for detecting experimental changes. It has been found, however, that mere removal of one adrenal is followed by gross changes in the appearance of cortical fat in the adrenal left in situ (Whitehead, 1933*b* (mouse); Simmons & Whitehead, 1937 (guinea-pig); Stilling, 1888 (rabbit)); the only sound method is therefore to take a group of animals as the unit and to compare the frequencies of the various appearances of fat in the adrenals of control and experimental groups.

SUMMARY

1. The histological distribution of fat and cholesterol in the adrenal cortex has been studied in 120 normal rats at various ages from birth to over 169 days.

2. Fat was almost invariably abundant in the outer half of the cortex; the fat content of the inner half showed wide but regular variations with age.

3. At birth the inner half of the cortex was fat-laden; at 14 and 29 days it was fat-free or virtually fat-free; at later ages it usually contained fat, which was sometimes abundant.

4. The range of variation in the appearance of cortical fat was the same in both sexes.

5. The fat content of the inner half of the cortex was on the average higher in the females than in the males at all ages after 14 days.

6. The appearance of cortical fat was closely similar in both adrenals of any rat.

7. There was no evidence of any relation between cortical fat and the oestrous cycle.

8. All the fat in all the adrenals contained cholesterol, the distribution of cholesterol being the same as that of fat.

9. The results are compared with those previously obtained on the normal mouse, guinea-pig and rabbit.

10. The significance of the results for the experimental study of cortical fat is discussed.

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EXPLANATION OF PLATE 1

Figs. 1–4 show transverse frozen sections, stained with Sudan IV without counterstain, of adrenals of normal rats.

Fig. 1. Showing the four zones of the cortex: (1) a narrow black zone (fat-laden) below the capsule, (2) a narrow light grey zone (no fat obvious), (3) a broad black zone (fat-laden), and (4) a light grey zone (no fat obvious) occupying the inner third of the cortex. Medulla lighter grey than surrounding cortex. Right adrenal of a 65 g. female aged 29 days. ×60.

Fig. 2. Zones 1 and 2 less obvious than in fig. 1. Zone 3 (black, fat-laden) extends to the inner border of the cortex, zone 4 being indistinguishable. Cortex and medulla interdigitate. Right adrenal of a 5·5 g. female under 1 day old. ×100.

Fig. 3. Zones 1 and 2 as in fig. 1. Zone 3 is relatively narrower and zone 4 correspondingly broader than in fig. 1. Left adrenal of a 23 g. male aged 14 days. ×85.

Fig. 4. Pattern as in fig. 1, zone 4 occupying inner half of cortex. Zone 4 contains fat (dark grey) and therefore contrasts sharply with the medulla (almost white). The fat in zone 4 is less abundant than in zones 1 and 3, both of which appear black. Right adrenal of a 186 g. male aged 84 days. ×60.

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The correct magnifications of figs. 1–4 (Plate 1) are 41, 69, 58, and 41 respectively.

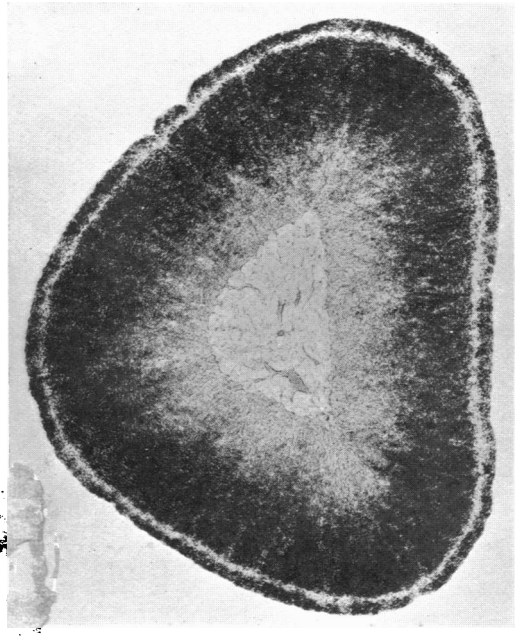

Fig. 1.

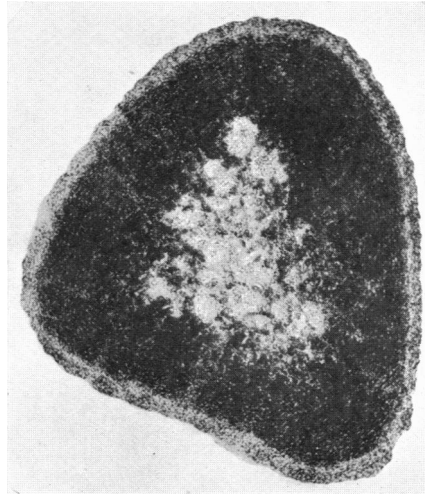

Fig. 2.

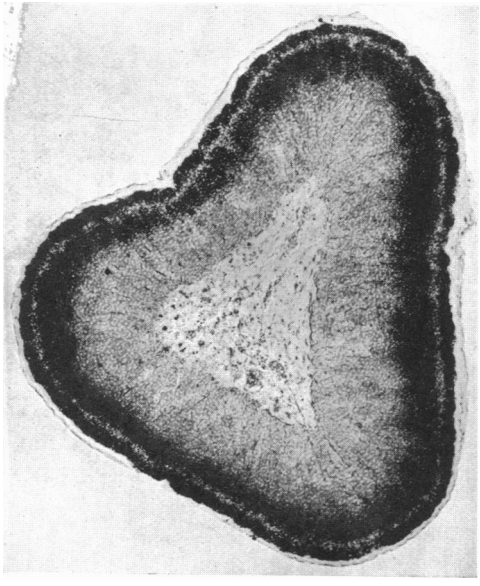

Fig. 3.

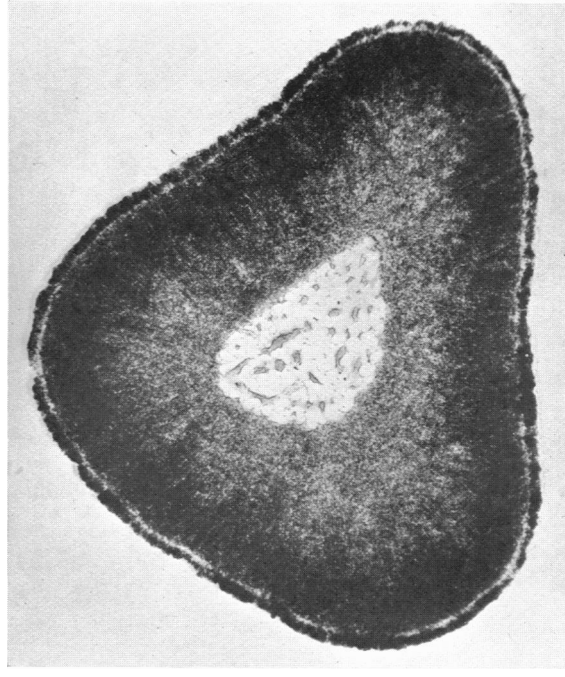

Fig. 4.