GROWTH AND MITOSIS IN THE MOUSE SUPRARENAL

By RAYMOND WHITEHEAD

From the Department of Pathology, Victoria University of Manchester

PART I. GROWTH

THE following measurements were made to determine approximately the rate of growth of the cortex and medulla of the mouse suprarenal, and to provide a rough standard for judging whether glands from experimental animals are broader or narrower than the normal.

MATERIAL

The suprarenals from mice of various colours were fixed in 5 per cent. potassium bichromate, to which an equal volume of 10 per cent. formol saline was added after about 2 hours. After remaining in this mixture overnight the glands were washed in water, dehydrated, cleared, and embedded in paraffin. Longitudinal sections were cut at 4μ, and a ribbon containing the central sections mounted. They were stained with haemalum and eosin. Measurements were made of 106 glands from 57 males, and of 113 glands from 61 females.

METHOD OF MEASUREMENT

A central longitudinal section of the mouse suprarenal is roughly ovoid, with a rounded end and a pointed end, from which the central vein emerges. In a previous paper (Whitehead, 1933) these were designated the paravenous and venous poles respectively. An imaginary straight line joining them was termed the major axis, and an imaginary straight line intersecting this axis at right angles midway between the poles was termed the minor axis. Three sections of each gland were measured along the minor axis. Thus the breadth of the cortex was found by averaging six measurements, that of the medulla by averaging three.

RESULTS

Explanation of figures

The results of the measurements are shown in four uniform figures. Ordinates denote breadths in mm.; abscissae, age of the mice in months. In figs. 1 and 2 each square denotes breadth of permanent cortex in one gland; in figs. 3 and 4 each square denotes breadth of medulla in one gland. Where two or more glands from mice of the same age group gave the same measurements the number of glands is indicated by the area of symmetrical rectangular blocks.

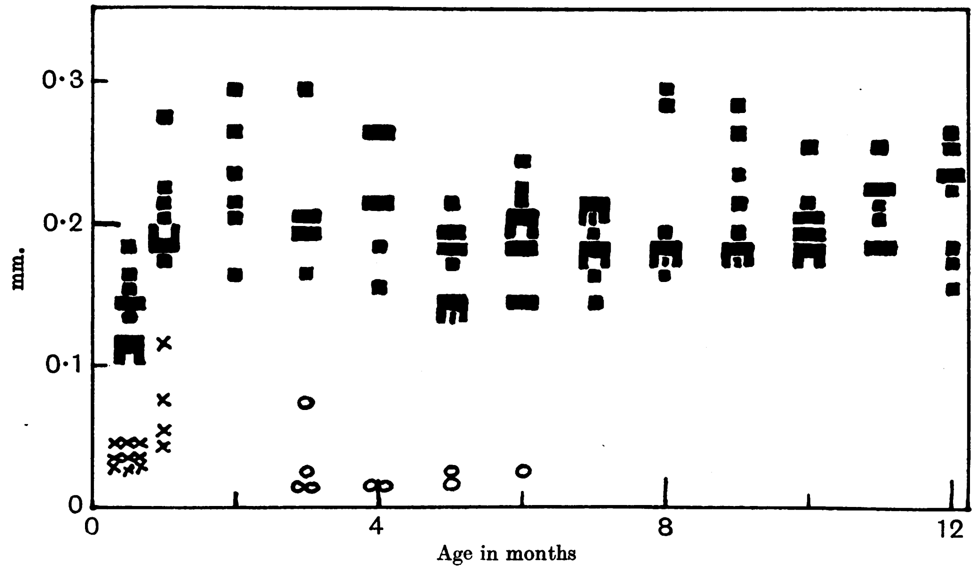

Fig. 1. Breadth of suprarenal cortex in male mice. Permanent cortex: squares; transitory cortex: crosses; perimedullary fibrous band: circles.

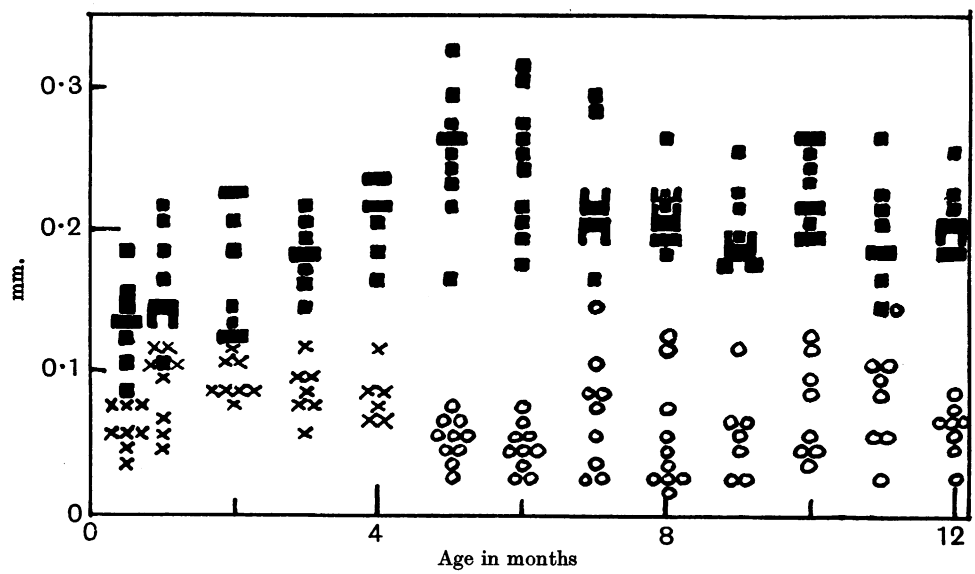

Fig. 2. Breadth of suprarenal cortex in female mice. Permanent cortex: squares; transitory cortex: crosses; perimedullary fibrous band: circles.

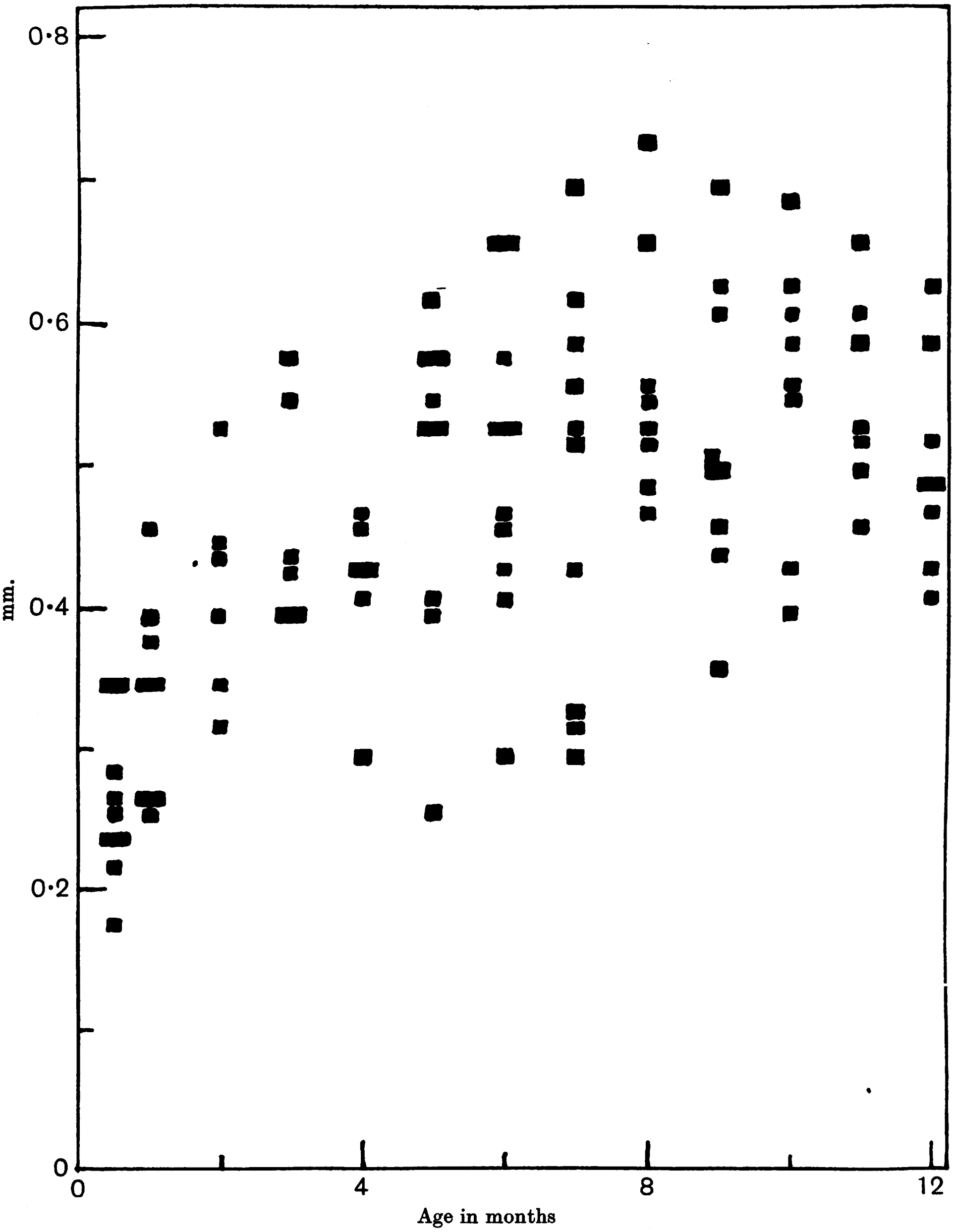

Fig. 3. Breadth of suprarenal medulla in male mice.

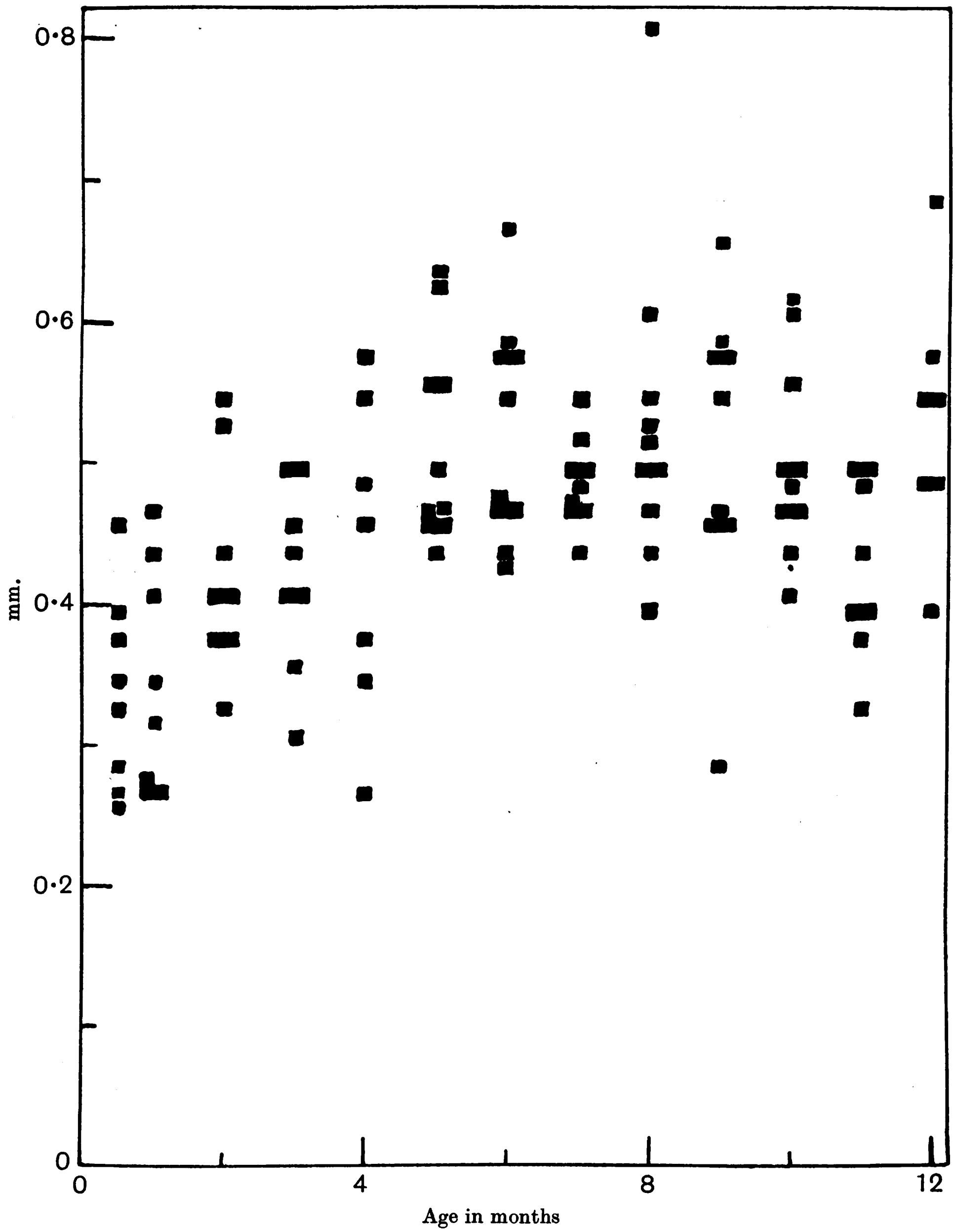

Fig. 4. Breadth of suprarenal medulla in female mice.

The lowest point of the middle of such a block marks the breadth. In figs. 1 and 2 the breadth of the transitory cortex (*X* zone) is indicated by crosses, that of the perimedullary fibrous band that succeeds the transitory cortex, by circles.

Breadth of permanent cortex

This is shown in fig. 1 for males and in fig. 2 for females. The average breadth of permanent cortex in both sexes is about 0·2 mm. This value is attained in males at 1 month, in females at 4 months. Before this epoch the female average is lower than the male average, but steadily rising. The breadth of permanent cortex varies widely within each age group. The range of variation is about the same at all ages and in both sexes.

Breadth of transitory cortex

The average breadth of transitory cortex is 0·03 mm. in males at 0·5 month (fig. 1, crosses). The variation is greatest at 1 month. The transitory cortex had disappeared from four of the eight glands measured at 1 month, hence the average is the same as at 0·5 month, viz. 0·03, although the range of variation is wider. In females the average breadth of transitory cortex attains 0·08 mm. at 1 month, remaining at this level until 4 months (fig. 2, crosses). The perimedullary fibrous band succeeding the transitory cortex is rarely of measurable breadth in males: in females, on the other hand, its average breadth is 0·06 mm. (fig. 2, circles).

Breadth of medulla

This is shown in fig. 3 for males and fig. 4 for females.

The average breadth of the medulla increases in both sexes until about 5 months. Thereafter it remains at about the same level in both sexes, viz. 0·5 mm. The range of variation in each age group is wide.

Discussion

Measurements

The minor axis, defined above, is the most suitable place for measurements of the mouse suprarenal, because the breadth of the cortex here varies least over unit distance. The cortex is rarely of the same breadth on both sides of the medulla, which as a rule appears to be placed eccentrically in the cortex. Sometimes a relatively thin shell of cortical tissue covers the medulla on one side of a longitudinal section. In this event the cortex on the other side is much broader. When the breadth of the cortex at both ends of the minor axis is averaged, the variations in a group of glands are much less than they would be if the cortex were measured at one end only of the minor axis in each gland. The phenomena noted are presented equally by permanent cortex, transitory cortex, and the perimedullary fibrous band that succeeds it.

The medulla is not always broadest at the minor axis. Its longest transverse diameter often occurs between the minor axis and the paravenous pole. It was desirable, however, to make all the measurements along one readily identifiable line, the minor axis. Hence the measurements of the medulla plotted in figs. 3 and 4 do not always represent the longest transverse diameter of the medulla. It would be difficult to determine accurately the total amount of medullary tissue present in any gland, for as much as one-half of the area of a central section of the medulla may be occupied by blood sinuses when these are dilated.

The measurement of three sections eliminates the risk of gross errors, which are sometimes made if only one section is measured.

Growth of the suprarenal

In both sexes permanent cortex increases in breadth as long as the transitory cortex is present. When the transitory cortex has disappeared there is no sex difference in the breadth of the permanent cortex, hence in the female it increases in breadth more slowly than in the male. Since the transitory cortex persists longer in the female than in the male, permanent cortex is broader in the male than in the female until the epoch at which female transitory cortex has disappeared.

The growth of the medulla shows the same time relations in both sexes, being complete at about 5 months. This identity is interesting as suggesting that there is no relation between the growth of cortex and medulla. If such a relation existed, the medulla might have been expected to reach its adult breadth at the same time as the permanent cortex in both sexes.

When the transitory cortex has disappeared, the only sex difference is the presence in the female suprarenal of a broad perimedullary fibrous band.

Summary of Part I

1. The permanent cortex of the mouse suprarenal increases in breadth in both sexes as long as the transitory cortex is present.

2. The average adult breadth of the permanent cortex in both sexes is 0·2 mm.

3. Since the transitory cortex lasts longer in the female than in the male, the permanent cortex increases in breadth more slowly in the female than in the male.

4. The medulla attains its adult breadth of 0·5 mm. at about 5 months in both sexes.

5. This identity of the growth phenomena of the medulla in both sexes suggests that the growth of the medulla is unrelated to the growth of the cortex.

6. When the transitory cortex has disappeared the presence in the female suprarenal of a broad perimedullary fibrous band is the only sex distinction.

PART II. MITOSIS

No systematic study of the natural frequency of mitoses in the suprarenals of the laboratory animals appears to have been made. The available literature, summarised by Jackson (1919), contains only casual observations.

The following study of the natural frequency and distribution of mitoses in the mouse suprarenal was made to furnish a basis for the interpretation of experimental findings and to determine the mode of growth of the transitory cortex.

Material and methods

The material described in Part I was used.

Mitosis counts were made of 107 glands from 57 males and of 115 glands from 61 females. Three sections of each gland were systematically swept for mitoses with a 1/12 oil immersion, and the situation and phase of each mitosis noted. Permanent cortex, transitory cortex and medulla thus yielded three values each. The highest of these was taken as the mitosis count.

Results

The mitosis counts for permanent and transitory cortex are shown in Tables I and II respectively.

Table I. *Natural frequency of mitoses in permanent cortex of mouse suprarenal*

Age (days)	Males					Average	Females						Average
15–17	16	28	22	26	.	19	18	9	29	–	13	.	16
	12	–	12	18	.		–	10	19	14	–	.	
28	9	6	6	13	.	8	19	18	–	18	5	2	9
	6	5	2	17	.		6	6	4	–	6	3	
57	–	2	2	.	.	2	17	26	29	0	.	.	15
	0	1	4	.	.		18	12	18	0	.	.	
85–86	2	1	2	–	3	2	0	0	0	0	.	.	0
	1	–	–	2	–		0	0	0	0	.	.	
111–112	2	1	0	.	.	1	2	0	0	1	.	.	1
	1	0	1	.	.		1	0	1	0	.	.	
141	0	0	0	1	0	0	1	–	0	1	1	.	1
	0	0	0	0	0		0	1	1	0	0	.	
167	0	0	1	0	1	1	0	0	1	1	1	.	0
	0	0	1	2	0		1	0	0	0	0	.	
201	2	0	1	2	0	1	0	0	0	0	1	.	0
	0	0	3	0	1		0	2	0	1	0	.	
227	1	0	1	0	.	1	0	0	0	0	–	.	0
	0	0	2	0	.		0	0	0	0	0	.	
256	0	1	3	1	1	1	0	0	0	0	0	.	0*
	0	0	0	2	1		0	0	0	0	0	.	
280	3	0	0	0	0	1	0	0	1	1	0	.	0
	0	0	0	0	4		0	0	2	0	0	.	
310	0	2	10	0	–	2	0	0	0	3	.	.	1
	0	·2	1	0	0		0	1	0	4	.	.	
336	5	1	0	2	.	3	3	0	1	0	.	.	1†
	8	1	0	4	.		1	1	2	1	.	.	

* 252 days. † 336–338 days.

Table II. *Natural frequency of mitoses in transitory cortex of mouse suprarenal*

Age (days)	Males				Average	Females						Average
15–17	19	12	13	9	14	4	8	5	–	7	.	7
	20	–	11	17		–	8	4	14	–	.	
28	0	–	–	1	1	4	20	–	3	1	0	4
	0	–	–	2		4	7	1	–	0	0	
57	.	.	.	.	.	4	2	1	1	.	.	2
						4	0	1	0	.	.	
85–86	.	.	.	.	.	0	0	0	0	.	.	0
						0	0	0	0	.	.	
111–112	.	.	.	.	.	0	0	0	0	.	.	0
						0	0	0	0	.	.	

Explanation of Tables I and II

Each entry represents one gland. For each age group of each sex there are two rows of figures. Those in the top row represent left glands, those in the bottom row the corresponding right glands. A cipher denotes a gland in which no mitoses were seen. A dash indicates an uncounted gland of a pair, or a gland from which transitory cortex had disappeared. The entries in Tables I and II correspond: e.g. in the left gland of the first male at 15–17 days permanent cortex contained 16 mitoses (Table I), transitory cortex 19 (Table II).

Mitoses in medulla

Mitoses in the medulla were rare. Two male glands at 15–17 days showed one each, one showed two. At 28 days, one gland showed one, one showed two. One gland at 111–112 days, one at 310, and three at 336, showed one each. One female gland at 15–17 days, three at 28, and one at 57, showed one each.

Frequency of different phases

The incidence of different phases of mitosis in the cortex is shown in Table III. The counts of all three sections of each gland, giving a total of 1660 mitotic figures, were used to determine this.

Table III. *Percentage incidence of different phases of mitosis in permanent (PC) and transitory (TC) cortex of mouse suprarenal*

Phase of mitosis	Males *PC*	Males *TC*	Females *PC*	Females *TC*	Average
Monospireme	59	52	51	42	51
Monaster	11	10	11	23	14
Diaster	3	3	2	5	3
Dispireme	27	35	36	30	32
Totals	100	100	100	100	100

Of the total eighteen mitoses seen in all sections of the medulla, nine were monospiremes, eight monasters, one a dispireme.

Distribution of mitoses

In the permanent cortex mitoses were commonest immediately beneath the capsule of the gland and rare elsewhere. They occurred throughout the transitory cortex. Comparison of counts from the same gland shows that the absolute number of mitoses in transitory cortex was usually less than that in the permanent cortex. The area of the part of permanent cortex where mitoses were frequent, however, exceeded that of the immature transitory cortex, and the actual frequency of mitotic figures in unit areas of both was about the same. Medullary mitoses were commonest at the periphery, but sometimes occurred elsewhere.

Absence of amitosis

No evidence of amitotic division was found. The resting nuclei of cortical cells appear circular or oval in section, and I have never seen irregular forms. The nuclei of the cortical stroma cells, however, though normally fusiform, vary much in size and shape. It is possible that some of these variants might be mistaken for nuclei of cortical parenchyma cells dividing amitotically.

Normal polar contouring

Hoerr (1931) observed that in guinea-pig suprarenals in which mitosis was active the cortical cells had convex inner borders and concave outer borders. This appearance, which he termed "polar contouring," is normally visible in the outer third of permanent cortex of the mouse suprarenal. It varies much in degree, but does not seem to depend on the number of mitoses present, since in the material here discussed it was less conspicuous in the immature glands than in adult glands showing few or no mitoses.

Discussion

Highest normal mitosis counts

Tables I and II show that after 2 months of age mitoses are rare in glands from both sexes. The highest count in permanent cortex in three sections of a gland exceeded four in only three instances among mice of both sexes aged 3–12 months, viz. in a male at 310 days (ten mitoses in one gland) and a male at 336 days (five and eight mitoses). The average values for the age groups giving the three high counts mentioned were two and three respectively.

Mitosis and growth

Table I shows that mitoses were numerous in both sexes while the permanent cortex was broadening. Mitoses were rare in males at 2 months, but still numerous in females. At 3 months they were rare in females. These results show that the immature permanent cortex increases in breadth by cell division at the periphery. Table II shows that mitoses were frequent in the male transitory cortex at 0·5 month and in the female transitory cortex up to 1 month. A few mitoses occurred in the female transitory cortex at 2 months: none were seen later.

Origin of transitory cortex

As shown above, in permanent cortex mitoses are rare except at the periphery, but occur throughout the transitory cortex. This demonstrates that transitory cortex increases in breadth by cell division and not by a change in the appearance of cells in the inner layers of permanent cortex. Whether transitory cortex cells are originally derived from permanent cortex cells is undetermined.

Frequency of spiremes

Table III shows that in permanent and transitory cortex of both sexes the phases of mitosis in order of decreasing frequency were monospiremes, dispiremes, monasters, and diasters. It is possible that some figures taken as monospiremes may have been members of dispiremes whose second members were not visible in the section, and also that some figures reckoned as dispiremes may really have been adjacent monospiremes, two adjacent cells chancing to be about to divide. The frequency of spiremes in the suprarenal was noted by Kolmer (1918) and Hoerr (1931) who attributed it to the long duration of the prophase. The fact could be more simply put by saying that the aster stages are more quickly traversed than the spireme stages.

Summary of part II

1. Mitoses were numerous in both permanent and transitory cortex of the mouse suprarenal while these were actively growing.
2. Mitoses in permanent cortex were commonest in the subcapsular layers of cells, rare elsewhere.
3. Mitoses occurred throughout the transitory cortex.
4. Mitoses were rare in the medulla.
5. The phases of mitosis in permanent and transitory cortex of both sexes were in order of decreasing frequency, monospiremes, dispiremes, monasters and diasters.
6. In adults the highest average number of mitoses was three: counts up to ten were found in single glands.
7. Polar contouring is normal in permanent cortex and its degree does not seem to depend on the degree of mitotic activity.
8. Permanent cortex increases in breadth by cell division at the periphery.
9. Transitory cortex increases in breadth by cell division, not by modification of permanent cortex cells.
10. No evidence of amitosis was found.

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