THE GROWTH CYCLE OF THE CELLS OF THE ADRENAL CORTEX IN THE ADULT RAT

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The experiments reported in this paper have been designed to test the claim that cells of the capsule of the adrenal gland become transformed into glandular elements, thus contributing new cells to the cortex to replace those disintegrating on completion of their life cycle. It seems generally agreed that the adrenal cortex of mammals shows centripetal migration of cells. Growth and differentiation take place in the outer parts of the cortex—the zona glomerulosa and the outer portion of the zona fasciculata; mature cells are to be found in the spongiocyte region of the zona fasciculata, while the inner zona reticularis is a place where cell death and disintegration occurs (Graham, 1916; Hoerr, 1931; Bennett, 1940). To this, Zwemer and his co-workers (Zwemer, Wotton & Norkus, 1938; Salmon & Zwemer, 1941; Wotton & Zwemer, 1943) have added the concept that a stratum of undifferentiated 'fibroblast-like' cells in the deeper part of the capsule normally elaborates cells which, by proliferation and migration, become glandular elements of the zona glomerulosa. It has further been suggested that the capsule plays an important part in regeneration of the gland after enucleation (Ingle & Higgins, 1939; Baker & Baillif, 1939).

Salmon & Zwemer (1941) studied the problem of transformation of capsular cells into glandular in animals stained *intra vitam* with trypan blue. They noted that cells in the capsule of the adrenal stored trypan blue in the form of fine granules after subcutaneous injection of the dye, and they then traced the subsequent fate of these marked cells. They concluded that, with increasing time interval after injection, there was a progressive inward movement of dye-containing cells until at 20–30 days after the first injection of dye, blue cells were to be found in the zona reticularis. Analysis of the data presented by Salmon & Zwemer raised some doubt as to the correctness of the interpretation that had been made. Not only did the amount of dye given to the animals vary, but so also did the period of time during which it was administered. Since, as Cappell (1929) has shown, vital dye is found in high concentration in the blood plasma for several days after a single injection, the time during which cells were exposed to the dye was considerable in the experiments reported. This indicates that it is difficult to determine just when any particular group of cells was first 'marked' by the dye. Further, in the experiments of Salmon & Zwemer, where dye-containing cells were observed in the zona reticularis 20–30 days after the first injection, it appears that injection of dye was continued throughout the whole of this time, and that the appearance might be, as Calma & Foster (1943) suggested, an expression of generalized absorption of dye by the cortical cells. Calma & Foster were unable to confirm the results of Salmon & Zwemer and in view of the difficulties in the acceptance of the latter's views, it was felt that their work should be repeated using a rigidly controlled technique. The amount of dye given required to be below the toxic level and to be constant with respect to the body weight of the animal. This predetermined amount should be given in one dose, thus ensuring that the 'commencement' of the experimental period can be dated as accurately as possible. Such an experimental series is described in this paper.

If it be true that the life cycle of the cortical cells begins in the capsule, and the cells then migrate inwards, it is possible that the mitotic activity of the capsular and subcapsular cells might give an indication of this.

Bearing in mind the observations of Graham (1916) and Hoerr (1931) that mitotic figures were most numerous in the outer part of the adrenal cortex in guinea-pigs, it seemed that determination of the numbers of these in the various cortical zones (including the capsule) of the rat, might be of value in two ways. First, determination of absolute values for dividing cells in the different cortical zones should give information regarding the relative amount of proliferation in each, especially if colchicine were used to arrest mitosis over a definite period of time. Secondly, such absolute values for normal rats would permit comparisons of the amount of cell proliferation in the adrenal cortex following experimental procedures. This second line of inquiry is not considered further in this paper. We know very little about the rate of cell division in the normal rat adrenal, and such information would be of obvious use when we consider how much the rat is used in endocrinological research.

Blumenfeld (1939) and Nathanson & Brues (1941) have reported on the number of dividing cells in the rat adrenal cortex. The former expressed his results in terms of mitoses per cubic mm. of cortex, while Nathanson & Brues (who worked with immature

* Aided by a grant from the Colston Research Fund.

female animals), gave their observations as the number of dividing cells per 10,000 cells counted. In the experiments reported here, the method of Nathanson & Brues has been amplified, absolute figures being given for the entire cortex, and for each zone of it, including the capsule.

MATERIAL AND METHODS

Twenty-six adult female albino rats of the Wistar strain were used in this work. All were kept under identical conditions of diet and housing. Special consideration was given to the temperature of the animal house, which was maintained at 70° F. to avoid any possible stimulant effect of low temperature on the adrenal cortex.

The trypan blue used for injection was made up in a 1 or 2 % solution in distilled water. It was filtered and stored in 2 c.c. ampoules, which were then sterilized. This was found to be a very convenient method of keeping the dye solution, and, contrary to what has been said by some (Romeis, 1932), no untoward effects were observed on injection of the dye solution thus made, 4 months or more after preparation. The animals were stained by a single intraperitoneal injection of either the 1 or 2 % solution of the dye in such amount that a dosage of 20 mg./100 g. body weight was given. It had been determined by preliminary experiment that this amount of dye was far below the toxic level and yet gave adequate staining of adrenal cortical cells.

Since Cappell's (1929) observations had shown vital dye to persist in the blood plasma for some time after a single injection, it was necessary, for the evaluation of our results, to investigate this point further. Accordingly an adult rat was given a single injection of the standard dose of trypan blue for its body weight. The blood plasma was examined at 24 hr. intervals afterwards. This was coloured deep blue until the 5th day, when a slight lessening of the colour was observed. On the 6th and 7th days this lessening of blue colour in the plasma became much more obvious. The experiment was then terminated. Although no quantitative estimations were made, it may be stated that a single intraperitoneal injection of trypan blue results in a uniformly high concentration of that dye in the plasma for 5 days; after that time the concentration diminishes fairly rapidly.

Animals were killed by ether at intervals from 12 hr. to 21 days after injection. The adrenals were fixed in Bouin's fluid, Heidenhain's Susa fluid, 10 % formol-saline or Bensley's formalin-Zenker. For preservation of dye-granules, the first two of these fixatives were found extremely good. Formalin-Zenker was quite useless. Paraffin sections were cut at 4–5 μ. When the object was to study dye granules, sections were mounted unstained, or lightly counterstained with Mayer's carmalum or safranin. Safranin gave very beautiful preparations in which the cell cytoplasm had a somewhat translucent appearance and the dye granules stood out in vivid contrast.

In the investigation of cell division in the adrenal cortex, two series of glands were used. One consisted of 14 adrenals from normal animals, and the other of seven glands from rats which had received colchicine in a dosage of 0·15–0·2 mg./100 g. body weight by subcutaneous injection 16 hr. before death. The colchicine must be freshly prepared, since in solution it undergoes rapid deterioration under the influence of light. The adrenals from both these groups were sectioned in paraffin at 5 μ and stained either with iron haematoxylin or Masson's trichrome stain (using light green).

OBSERVATIONS

(1) *The reaction of the cortical cells of the rat adrenal to intravitam trypan blue*

The reactions of the various zones of the cortex will be described separately, and for the purposes of this paper, the capsule is considered as a cortical zone.

Capsule. The adrenal capsule in the adult rat consists of some 4–6 layers of cells. The most peripheral are elongated and spindle-shaped in transverse section, and, as one passes towards the zona glomerulosa, the cells become shorter and plumper, the cytoplasm increasing in amount. A variable number of macrophages is found in the outer layers of the capsule.

Capsular cells commenced to segregate trypan blue a few hours after its introduction into the circulation of the animal. Twelve hours after injection a few, small, pale blue granules were visible in a number of the fibroblast-like cells here. Much larger pale blue granules could be seen in the macrophages at this time. The granules became more intense in colour, and rapidly increased in number. At 48 hr. the entire capsule was full of cells exhibiting these small, blue granules (Pl. 1, fig. 1). In all the cells of the capsule, except in the macrophages, the dye particles were characteristically small and of uniform size. Once established in the cell cytoplasm, they persisted throughout the experimental period, being found at all times up to 21 days after injection.

Glomerular zone. The glomerular cells reacted to trypan blue by segregating the dye in the form of very fine granules, exactly similar to the granules seen in the majority of the capsular cells. The segregation process commenced during the first 24 hr. after injection and seemed to occur with equal intensity at any level in the zone. Thus, at 30 hr. after injection, some glomerular cells as deep down as the outer border of the zona fasciculata showed dye in their cytoplasm, and at 48 hr. cells of the entire glomerular zone contained these granules (Pl. 1, fig. 1). In the preparations studied there was no suggestion of an inward spread of the

dye from the periphery of the adrenal cortex. Segregation of it occurred in a similar manner in all the glandular cells and about the same time after injection. In cells undergoing mitosis it was noted that the dye granules were dispersed in approximately equal numbers to the extremities of the spindle, thus presumably behaving as inert cytoplasmic structures.

Fascicular zone. Intra vitam trypan blue was not constantly observed in cells of the zona fasciculata. Most commonly the dye was found in cells at the outermost part of this cortical zone (Pl. 1, fig. 5), where small granules were found fairly evenly distributed throughout the cytoplasm of some of the cells. They resembled the granules in the cells of the glomerular zone and the fibroblast-like cells of the capsule, but differed in that they appeared somewhat later, about 4 or 5 days after injection. But, at this time, the concentration of trypan blue in the blood plasma was still high (see above).

Trypan blue was sometimes noted in cells in the deeper part of the zona fasciculata (Pl. 1, fig. 4). In these, the granules were much coarser than in the cells of the outer cortical zones. They therefore resembled macrophages, and their association with cortical sinusoids, as well as other micro-anatomical characters, left no doubt that they were phagocytic cells of the sinusoidal endothelium.

Reticular zone. A number of cells in the reticular zone constantly contained dye during the experimental period.

The first type recognizable (Pl. 1, fig. 3; Pl. 2, fig. 9) was an irregular cell whose nucleus was hyperchromatic, deformed and located at one side of the cell body. The cytoplasm contained vital dye along with masses of pigment. The amount of vital dye taken up by the cell seemed to vary inversely with the amount of pigment present. This dye was not arranged regularly in the cytoplasm, but appeared as masses of varying size between the pigment granules. These cells conformed in other characters with the 'dark' cells of the zona reticularis described by Hoerr (1931).

A second type of dye-containing cell constantly found in the zona reticularis is illustrated in Pl. 1, fig. 2. It was found to be characteristic of this cell that trypan blue had entered into it, and stained the cytoplasm in a *diffuse* manner. Sometimes even the nucleus took up the vital dye. In the group of cells figured there are all stages between mere coloration of the cytoplasm by trypan blue to intense staining of the nucleus along with vacuolar degeneration of the cytoplasm. For reasons which will be discussed later, these cells must be considered as dying or dead.

(2) *Cell division in the normal adrenal cortex*

As already noted, the number of mitotic figures observed in each cortical zone was determined in relation to the total number of cells counted in that zone. Tables 1 and 2 give the results obtained from two series of animals; the first consisted of normal individuals, while the rats in the second group had received colchicine.

Table 1. *Mitotic counts in adrenals from normal rats. Expressed as dividing cells per* 10,000 *cells counted*

Animal no.	Capsule	Glomerulosa	Fasciculata	Reticularis	Total cortex
194A	17	23	0	0	10·6
194B	15	17	33	14	18·9
195A	33	17	0	19	16·7
195B	19	10	7	7	10·1
196A	25	46	20	16	26·2
196B	16	24	38	7	22·1
199A	20	21	8	0	11·8
199B	17	16	6	0	8·8
203A	11	0	13	9	9·1
203B	0	16	24	0	10·0
204A	7	8	0	0	3·6
204B	0	0	6	6	3·3
205A	0	22	7	0	7·1
205B	7	16	17	0	10·0
Average	13·3	16·8	12·7	5·6	12·0

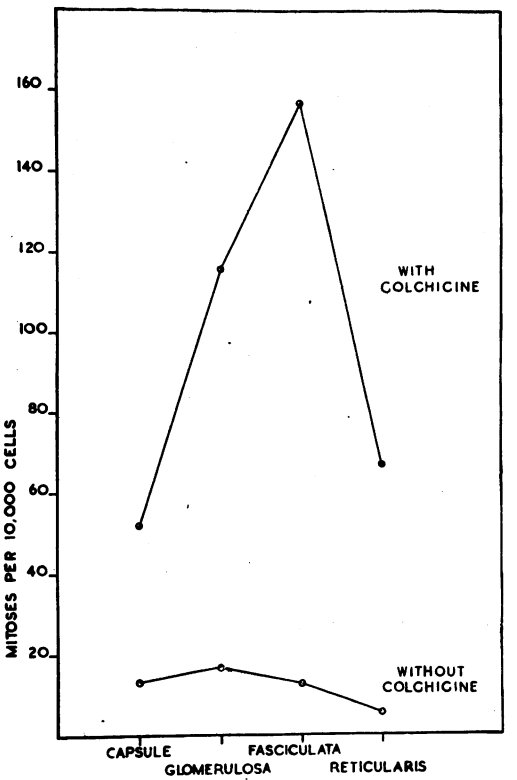
Table 2. *Mitotic counts in adrenals from colchicine-treated rats. Expressed as dividing cells per* 10,000 *cells counted*

Animal no.	Capsule	Glomerulosa	Fasciculata	Reticularis	Total cortex
208A	45	46	83	46	64
208B	54	89	133	37	80
233A	56	151	231	93	133
233B	25	169	212	82	118
238A	62	105	143	53	91
240A	66	104	120	48	86
242A	55	152	179	102	124
Average	52	116	157	66	99

It will be seen from Table 1 (data from fourteen adrenals) that the mitotic count for the total cortex varied, in the normal animal, between 3·3 and 26·2 per 10,000 cells. The average for the series was 12·0. In the individual zones of the cortex there was variation between different animals and even between the two glands from the same animal. Dividing cells were most numerous in the zona glomerulosa where the average for the series was 16·8 per 10,000. They were rather less frequent in the capsule (average, 13·3), and slightly less frequent still, in the zona fasciculata, where the average was 12·7. These figures are graphically represented by the lower curve in Text-fig. 1.

For the second series it was calculated from the data of Brues & Cohen (1936) that 0·15–0·2 mg. of colchicine per 100 g. body weight would be effective in arresting cell division over 16 hr., and the adrenals from rats thus treated showed many more mitoses than normal. Thus, in Table 2, we find the

average number of mitoses for the whole cortex to be 99 per 10,000 cells. The variation is not so marked as in the normal animals, ranging from 64 to 133. The highest mitotic rates were found in the zona fasciculata; the zona glomerulosa came next, while in the zona reticularis and the capsule the rates were considerably lower (Text-fig. 1, upper curve).



Text-fig. 1. Frequency curves of mitoses for the cortical zones of the adrenal in normal and colchicine-treated rats.

It is worth noting that the distribution of mitoses in the various cortical zones was not uniform in the adrenals studied. To illustrate this, camera lucida tracings (Text-figs. 2, 3) were made from sections through the centre of the adrenal in two colchicine-treated rats, and the positions of dividing cells plotted. In Text-fig. 2 there is great activity in one-half of the section while the other is relatively quiescent. In the active portion of the gland mitoses are numerous at all levels in the cortex, and it seems possible that the call for production of new cells varies in different parts of the same gland. There is, of course, nothing new in the concept of differential activity in various parts of one gland, but it does not hitherto seem to have been noted in the adrenal cortex.

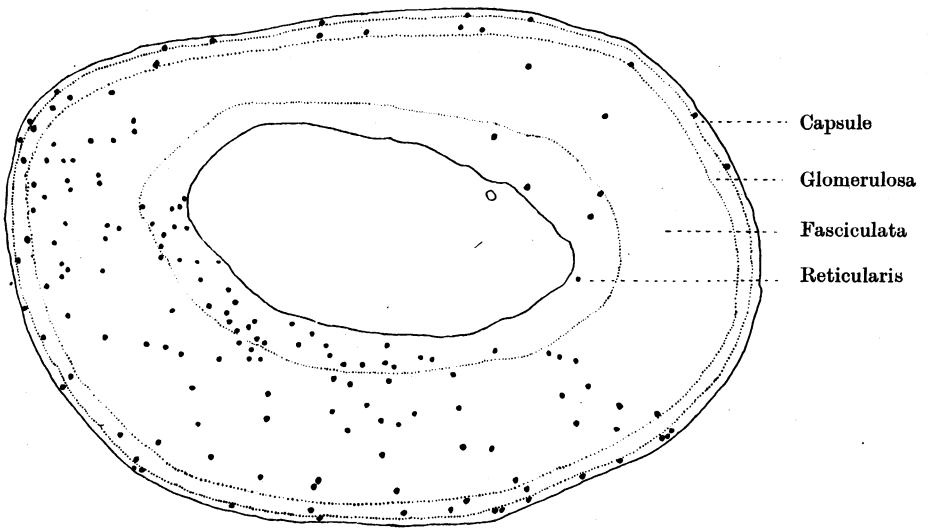
Text-fig. 3 demonstrates the conditions found in another animal. Here, mitoses are seen at all levels in the cortex, and in all parts of the section, but there is a tendency for them to be located more in the superficial than in the deeper parts of the cortex.

It is noteworthy that mitoses were observed in the zona reticularis in a considerable number of normal animals, and constantly in the animals treated with colchicine. Examples of such mitoses are shown in Pl. 1, figs. 2, 3, and Pl. 2, fig. 8. These are in glandular cells, and multiplication of endothelial and connective tissue elements (which occasionally is found in normal adrenals) has been disregarded in compiling Tables 1 and 2.

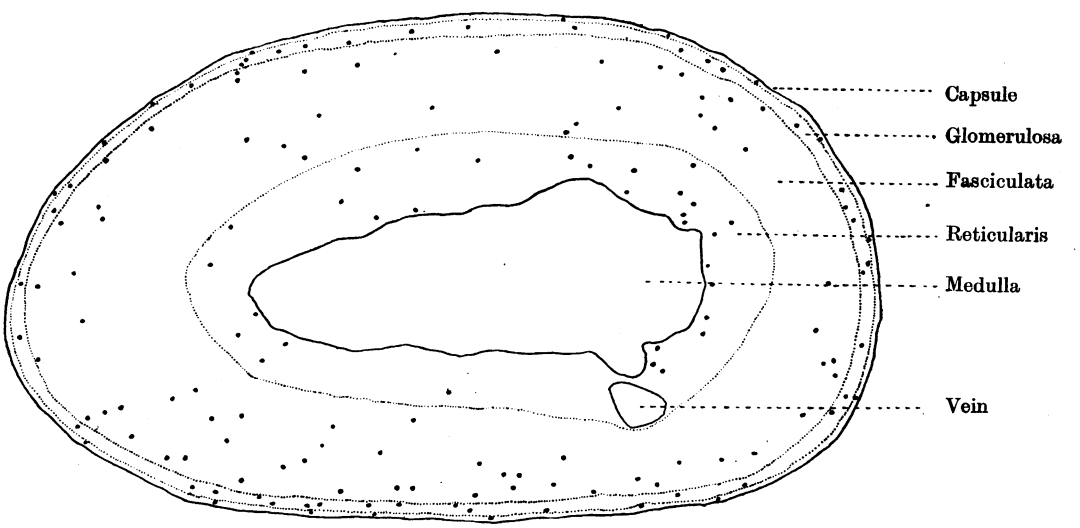
Mitotic figures in the adrenal capsule are shown in Pl. 2, figs. 6, 7, and it will be seen that they occupy a position in the capsule just external to the zona glomerulosa. It was not common to observe mitoses in the more superficial flattened cells of the capsule. Sometimes it was difficult to determine whether a dividing cell in the deeper region of the capsule should properly be assigned to that zone or to the zona glomerulosa. At some places (Pl. 2, fig. 7) there were appearances suggesting that cell cords of the zona glomerulosa were connected with, and taking origin from, germinal foci in the deep part of the capsule.

DISCUSSION

The observations on animals stained *intra vitam* do not support Salmon & Zwemer's contention that the administration of vital dye provides a *method* whereby the derivation of adrenal cortical cells from the capsule may be demonstrated. I do not deny the possibility of such an origin for zona glomerulosa cells, but submit that this method does not demonstrate it. In the present experiments, pale blue granules were observed in cells of the adrenal capsule 12 hr. after injection of dye; similar granules were visible at all levels in the zona glomerulosa 24–30 hr. after injection, while at 48 hr. the segregation of trypan blue by the cells of both capsule and glomerulosa was very evident (Pl. 1, fig. 1). It is extremely difficult to believe that, in this short time of 24 hr., such a number of dye-containing cells in the zona glomerulosa could have been derived from similar dye-storing cells of the capsule. The appearances suggest, on the contrary, that the fibroblast-like cells of the capsule, the cells of the zona glomerulosa and the cells of the outermost part of the zona fasciculata react simultaneously in a similar fashion to *intra vitam* trypan blue which is present in high concentration in the blood plasma during the whole of this period. In brief, the fact that the dye particles in all these cells (*a*) are morphologically similar, (*b*) become microscopically visible within 24 hr., and (*c*) show little evidence of diminution in number as one progresses inwards from the capsule, indicates that there is a certain functional affinity between these peripheral cortical cells, but no more than that. Calma & Foster (1943),



Text-fig. 2.



Text-fig. 3.

Text-figs. 2, 3. Camera lucida drawings of sections through the middle of the left adrenal of two adult rats treated with colchicine 16 hr. before death. The different zones and the sites of mitotic figures are shown.

repeating the experiments of Salmon & Zwemer (1941) for rats, were unable to satisfy themselves that the method yielded any evidence for centripetal growth of cells in the adrenal cortex. Similar findings were noted in the mouse by McPhail (1944).

The reactions to trypan blue shown by the cells of the zona reticularis do not seem to have been reported hitherto. Certainly, neither Cappell (1929) nor Calma & Foster (1943) mention them, and Salmon & Zwemer only saw dye-containing cells in the zona reticularis after 20–30-day periods of continuous injection. In the present experiments these *intra vitam*-stained cells were seen in the deepest zone of the adrenal cortex in all the animals studied. That an acid azo dye such as trypan blue, injected *intra vitam*, will enter into and stain diffusely the cytoplasm of degenerating cells has been known for some time. Ludford (1933) has discussed this phenomenon in his review of vital staining. He considers that diffuse cytoplasmic staining, with or without *intra vitam* staining of the nucleus, indicates cell senility and death; further, he believes that the degree of diffuse staining (in chronic experiments) is a measure of the decay of the cell. Relevant to this, Darlington (1937) perfused surviving organs with relatively high concentrations of trypan blue (1/4000 in Ringer-Locke solution) to detect cell death, while Bennett (1940) injected large amounts of vital dye for the same purpose in acute experiments. In both these procedures only the nuclei of dead cells were coloured by the dye. Pl. 1, fig. 2 shows cells in the juxta-medullary zone of the rat adrenal which have stained diffusely with trypan blue following a single injection of the dye. The morphological characters of these cells show that they are degenerate or dying. Their presence in the deeper part of the cortex indicates the undoubted occurrence of cell death in this zone, but since in addition mitoses are often observed in glandular elements here (see Pl. 1, figs. 2, 3 and Pl. 2, fig. 9), it is inaccurate to regard the zona reticularis of the rat adrenal as purely one of cell degeneration.

The cells of the zona reticularis that contain pigment and also take up vital dye must next be considered. Hoerr (1931) identified pigmented cells in the zona reticularis in the guinea-pig (where they are especially numerous) as the well-known 'dark' cells of this region. He came to the conclusion that they were glandular elements well on the way to degeneration. Blumenfeld (1939) has noted pigment cells in the zona reticularis of the rat adrenal, and described their increase in number and size after ovariectomy. Their nuclei resembled those of the cells lining the sinusoids, and this, with other evidence at his disposal, led him to believe them to be derived from the sinusoidal endothelium and to be phagocytic in nature. It has proved very difficult to decide in the normal material studied by the writer, whether the pigment cells of the rat adrenal are transformed glandular elements, or whether they belong to the reticulo-endothelial system. The fact that they are found in that part of the adrenal cortex where cell degeneration is undoubtedly taking place suggests their association with this process. They take up trypan blue in irregular coarse masses, thus resembling macrophages more than anything else, and indeed are not unlike the pigmented macrophages described by Rossman (1942) in association with the regressing corpus luteum of the macaque monkey. While it seems most probable that pigment cells in the rat adrenal cortex are mainly, if not all, reticulo-endothelial cells, the evidence available is not sufficient to enable one to pass final judgement on their nature.

The location of the majority of capsular mitoses in a layer of cells adjacent to the zona glomerulosa strongly suggests that some glandular elements, at least, are derived from these deep capsular cells. Additional evidence for this was seen in a number of sections (Pl. 2, fig. 7) where cell columns of the zona glomerulosa appeared to be in connexion with germinal foci in the capsule. Salmon & Zwemer (1941) refer to these deep capsular cells as a 'pre-glomerular zone' in their *intra vitam* stained animals. Our study of dividing cells in the rat adrenal capsule lends support to the concept of such a germinal zone for the gland proper.

The fact that mitoses occur with a higher frequency in both the zona glomerulosa and the zona fasciculata than in the capsule, need be no hindrance to accepting the view that some new glandular cells arise from the pre-glomerular zone. If such a cell divides, and a daughter cell is transported inward into the zona glomerulosa, it will probably continue to divide for a time depending on the need for new cortical cells—since it is commonly accepted that cells of the zona glomerulosa are functionally immature (Bennett, 1940).

The absolute values for mitoses in the adrenal cortex for the series of animals reported here may be compared with figures given by Nathanson & Brues (1941) for immature female rats, 4½ weeks old. These workers were primarily concerned with the effect of testosterone on the mitotic rate in the rat adrenal cortex, but they give data for controls. Values of from 23 to 42 mitoses per 10,000 cortical cells were found by them in animals to whom colchicine had been given. These values are lower than those reported here (99 mitoses per 10,000 cells of the entire cortex) but this may be due to differences in technique. Thus the dose of colchicine used by Nathanson & Brues was 0·1 mg./100 g. body weight; the time of death was never more than 12 hr. after administration of the drug. The maximum effect seems, from their figures, to have been obtained 4–10 hr. after giving the alkaloid. In the series reported here, on the other hand, both

the dose of colchicine (0·15–0·2 mg./100 g. body weight), and the time interval between injection and death of the animal (16 hr.), were greater. Brues (1936) claims that the number of mitoses seen in arrested metaphase after administration of colchicine over a period of time is equal to the number of mitoses which would normally have occurred and gone on to completion during that time. Brues & Cohen (1936) state that the time period when colchicine exercises its effect on dividing cells is from 6 to 18 hr. after injection, the optimum dose being 0·1–0·2 mg./100 g. body weight. This means that the higher dosage and longer period of action of the drug in our series might well account for the higher figures obtained.

As far as the age factor is concerned, since it is usually assumed that young rats show a higher mitotic frequency than adults, one would have expected the counts of Nathanson & Brues on 4½ weeks-old rats to be distinctly higher than those in the present experiments, in which adult rats have been used. That this is not the case calls for an explanation which cannot yet be given.

There is a point concerning the identification of mitoses arrested by colchicine which must be stressed. The typical colchicine-arrested mitosis shows the chromosomes dispersed throughout the cytoplasm in a characteristic manner. Such cells are easily identified; but in dividing cells which have been subjected to the action of colchicine for relatively long periods of time, the chromosomes are clumped. Examples of such mitoses are given in Pl. 2, figs. 7, 8, and unless well-differentiated iron haematoxylin preparations are studied under the oil-immersion objective many of these mitoses may be missed.

SUMMARY AND CONCLUSIONS

1. The reactions of the adrenal cortical cells to *intra vitam* trypan blue have been investigated in a series of rats.

2. Most of the cells of the peripheral part of the cortex, that is, the capsule, the zona glomerulosa and the extreme outer part of the zona fasciculata segregate trypan blue in the form of minute, intracytoplasmic granules; this commences during the first 24 hr. after injection, and reaches a maximum at 48 hr. There is no evidence that dye-containing cells migrate from the capsule into the zona glomerulosa: the whole region seems to react as a functional unit. The contention of Salmon & Zwemer (1941) that *intra vitam* staining of the adrenal provides a method for demonstrating the origin of glandular elements from the capsule has not been supported by the present work.

3. Certain cells related to the capillaries in the inner part of the zona fasciculata store *intra vitam* trypan blue in the manner characteristic of macrophages.

4. In chronic experiments, dead and senescent cells in the zona reticularis stain *intra vitam* in a *diffuse* fashion. Their cytoplasm, and in the case of dead cells, their nuclei, appear uniformly blue. The pigment cells of this zone segregate trypan blue like macrophages, and are believed to be reticuloendothelial cells, not glandular elements. The number of both these cells in the adrenal cortex is an index of the amount of cell destruction in the gland.

5. Figures are presented for the rate of cell division in the cortical zones in animals treated with colchicine, and in normal ones. There is evidence that certain cells in the deeper part of the capsule give rise to zona glomerulosa cells by mitotic division. These cells may thus be termed 'preglomerular' as was done by Zwemer and his coworkers. Attention is also drawn to the presence of mitotic figures in glandular cells of the zona reticularis. In the rat, at least, this zone is one where cell degeneration and new formation normally proceed side by side.

It is a pleasure to acknowledge my indebtedness to Prof. J. M. Yoffey, who suggested this problem to me in the first instance, and whose continued interest and advice have been of great assistance. I wish to thank Miss D. Davidson for her careful work in the preparation of the coloured Plate and Mr J. E. Hancock for the photomicrographs.

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EXPLANATION OF PLATES

PLATE 1

(These figures are all drawn at a magnification of ×1050.)

Fig. 1. Capsule and zona glomerulosa 48 hr. after a single intraperitoneal injection of trypan blue. The cells of these two zones have stored the dye in the form of fine granules. A macrophage with coarser granulation is seen in the outer part of the capsule. A glomerulosa cell in metaphase shows dye particles concentrated at the opposite poles of the cell. Safranin stain.

Fig. 2. Juxta-medullary zone 6 days after a single dose of trypan blue. The medulla is to the left-hand side of the field. A number of cells have taken up the dye in a diffuse fashion, and in one, the nucleus has been stained. These are dead or dying cells. Note the mitotic figure in the bottom right-hand corner of the field. Safranin stain.

Fig. 3. Zona reticularis. Note the presence of a number of pigment cells with shrunken, hyperchromatic nuclei. Two fuchsinophil cells are to be seen, and there is also a cell in mitosis. Masson trichrome stain.

Fig. 4. Dye-storing cells in the wall of a sinusoid of the inner fascicular zone. The dye granules here are definitely larger than those found in the cells of the capsule and glomerulosa and approach those of the macrophage in size (cf. fig. 1). Safranin stain.

Fig. 5. Outer fascicular region 21 days after administration of trypan blue. The cells of this part react to the dye in the same way as do those of the capsule and glomerulosa. The number of granules in each cell is, however, less. Safranin stain.

PLATE 2

Fig. 6. Capsule and zona glomerulosa showing mitoses after colchicine. The periphery of the gland is towards the left. One mitosis lies in the 'pre-glomerular' region; a second is in the zona glomerulosa proper. Iron-haematoxylin stain. ×600.

Fig. 7. Capsule, zona glomerulosa and part of the zona fasciculata from a rat treated with colchicine. There are two mitoses in the 'pre-glomerular' part of the capsule (upper part of photomicrograph); from the area between them a cord of gland cells extends into the zona glomerulosa. There is an example of the contracted type of colchicine mitosis in the zona fasciculata towards the bottom of the photograph. Masson trichrome stain. ×600.

Fig. 8. Cortico-medullary junction of the adrenal from a colchicine treated rat. A mitotic figure with clumped chromosomes is seen about the middle of the photograph. A fuchsinophil cell (early degeneration) lies to its right, while some pyknotic nuclei of more frankly degenerate cells are below and to the left. Masson trichrome stain. ×600.

Fig. 9. Pigment cells (which appear dark in the photomicrograph) from the zona reticularis of a normal rat adrenal. Iron-haematoxylin stain. ×600.

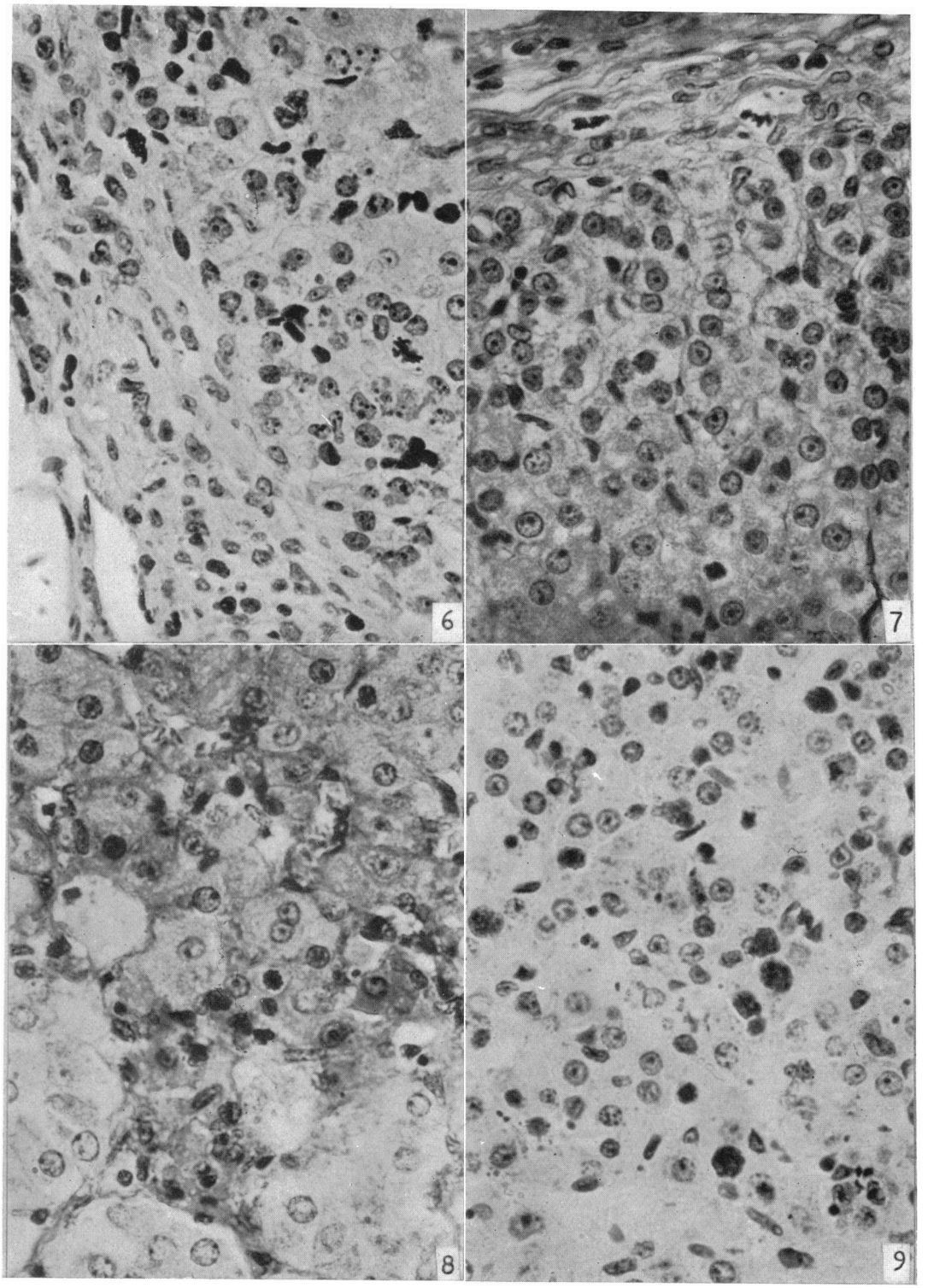

1


2


4


3
1/50 mm.


5
D.D.



BAXTER—THE GROWTH CYCLE OF THE CELLS OF THE ADRENAL CORTEX IN THE ADULT RAT