THE INVOLUTION OF THE TRANSITORY CORTEX OF THE MOUSE SUPRARENAL

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THE cortex of the mouse suprarenal has two components: an outer part, showing no gross changes with age, which may be termed the permanent cortex; and a part immediately surrounding the medulla, histologically distinct from the permanent cortex, which is transitory. The transitory cortex has been studied in detail by two previous workers. Miller (1927), who termed it the *X* zone, found that it first became evident in both sexes at 14 days after birth. In males it had disappeared at 38 days; in females, however, it continued to grow, gradually disappearing by a fatty change, so that at 80 to 200 days glands from males and females were indistinguishable. Deanesly (1928) confirmed and supplemented the observations of Miller (1927), describing the development of a new cortical zone after the disappearance of the *X* zone.

In the present paper attention is confined to the involution of the *X* zone, the conclusions reached differing slightly from those of both Miller and Deanesly.

MATERIAL AND METHODS

Two hundred and thirty glands were taken from 119 mice of various colours, 58 males supplying 112 glands, 61 females supplying 118 glands. The ages of the mice ranged from 15 to 338 days. All glands were fixed in 5 per cent. potassium bichromate, to which an equal volume of 10 per cent. formol saline was added after 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. Additional sections from some glands were stained by Weigert's stain for elastic tissue.

RESULTS

1. *The involution of the male X zone*

All glands at 15 to 17 days showed a healthy *X* zone. It was involuting in all at 28 days. In two pairs only a few *X* zone cells remained, and these were separated from the permanent cortex by a very thin sheet of fibrous tissue (fig. 1). The other two pairs of glands showed broader *X* zones, but signs of the separation of *X* zone from permanent cortex were obvious in each

of these four glands. In the glands studied the individual cells of the *X* zone appeared to be normal, no degenerative changes being seen in either nuclei or cytoplasm. At 57 days and at all the greater ages the male glands had the adult appearance. There was no trace of the *X* zone. The permanent cortex extended internally right up to the fine vascular fibrous tissue ensheathing the medulla, which had a perfectly smooth border (fig. 2).

2. *The involution of the female X zone*

At 15 to 17 days the female *X* zone was identical in appearance with that in the coeval male glands. Interdigitation of the *X* zone and medulla was more conspicuous than in males, but was not marked. At 28 days the *X* zone was broader than in the males, and no fibrous tissue could be seen separating *X* zone from permanent cortex. At 57 days two glands showed a few clear spaces. All the other glands at 57 and 85 to 86 days showed healthy *X* zones. At 111 to 112 days the *X* zone showed various phases of involution. One gland showed normal *X* zone on one side of the medulla, with interdigitation (fig. 3), whereas on the other side of the medulla in the corresponding situation there was an open network exactly like that seen in mature female glands from which the *X* zone has disappeared (fig. 4). The cells were fewer in number and the blood spaces wider than on the side where the *X* zone was normal. The edge of the medulla was smooth. The transition from normal *X* zone to the involuted area was quite gradual. The other gland of the pair showed the same features. A second pair of glands resembled this pair. A third pair of glands showed the *X* zone in an intermediate state. The *X* zone and medulla interdigitated slightly, and there was no fibrous band between them. The blood spaces were wider than in a normal *X* zone, and the nuclei of the *X* zone cells were smaller and stained more deeply. The last pair of glands showed a degenerate *X* zone outside a definite fibrous band. Within this a few healthy *X* zone cells were present. No evidence of involution by fatty change was found in this group of glands. At 141 days the *X* zone was absent. In one pair of glands a few small dark *X* zone cells could be seen lying between the fibrous band externally and the large dark cells of the medulla internally. At 167 days the *X* zone was absent from all; at 201 days, present in one pair; at 227 days, in one pair; at 252 days, in two pairs; at 280 days, in one pair; at 310 days, in one pair; at 336 to 338 days, in none.

3. *Basophilia of X zone cytoplasm (Miller, 1927)*

Miller (1927) found that the cytoplasm of the cells of the *X* zone stained definitely with haematoxylin after bichromate fixation. No definite haemalum staining of the cytoplasm of these cells was seen in any of the glands described above. Perhaps the result depends on the use of different methods of bichromate fixation. It is difficult to judge the colour of the cytoplasm because the strongly basophil nuclei of the *X* zone are so closely packed. Examined under a low power the whole *X* zone has a bluish tint.

DISCUSSION

1. *The involution of the X zone*

In the males the *X* zone had begun to involute at 28 days and had disappeared at 57 days. In the females, involution of the *X* zone was most frequent in the group at 111 to 112 days, an observation in fair agreement with that of Miller (1927), who found the highest incidence of degenerating *X* zones in a group whose age mid-point was 93 days. There is, however, a wide variation in the time at which the female *X* zone degenerates, and the factors on which this variation depends need elucidating. Miller (1927) noticed a similar variation, and suggested that it "might be lessened by breeding from one carefully standardized stock at one time."

The presence in single glands at 111 to 112 days of all stages from normal *X* zone to the fibrous band that replaces it in the adult, with no signs of the fatty change in the intermediate areas, indicates that the female *X* zone may sometimes degenerate without undergoing the fatty change as it does in the male. Involution without fatty change was observed by Miller (1927); and Deanesly (1928), who also saw both types, concluded that "in most glands studied, however, the *X* zone seems to disappear gradually as the result of an inconspicuous process of cell degeneration, which may begin either at the outside of the *X* zone or next the medulla." According to Deanesly's work and mine, therefore, involution of the female *X* zone without fatty change is the commoner mode.

In the males described above, the first sign that the *X* zone had begun to involute was the appearance of a very thin line of fibrous tissue between permanent cortex and *X* zone. This line became broader as the *X* zone cells disappeared, finally ensheathing the smooth-edged medulla of the adult gland. In the female, according to Miller (1927), "before or during the beginning of the degenerative process in the *X* zone, a regular connective-tissue line is laid down between the *X* zone and the rest of the cortex." This appearance was not seen in the glands described above, nor is it visible in any of Miller's (1927) figures illustrating the degenerative process. It can be seen, however, in the gland shown in Plate 14, Fig. 12, of Deanesly's paper. In her Fig. 13, "showing a later stage of degeneration of the *X* zone," there is no trace of such a connective-tissue line. It seems probable, therefore, that its occurrence is exceptional.

2. *The perimedullary fibrous band*

The appearances seen in the glands described above could be most simply explained by supposing that the perimedullary fibrous band replacing the *X* zone in the adult is formed, not by the deposition of new fibrous tissue outside the *X* zone, but by the shrinkage of the *X* zone stroma after the *X* zone cells have disintegrated. This would account for the occurrence of cells that I regard as *X* zone cells within the meshes of the uncontracted perimedullary

fibrous band in degenerating *X* zones. The congestion often seen would be due to dilatation of the pre-existing blood spaces owing to lack of the support that the *X* zone cells previously afforded. Deanesly (1928), who also believed that "a remnant of cells from the original *X* zone may persist among the fibrous tissue and outside it," stated that "during the resorption of the *X* zone the reticular fibrous tissue, which appears to be derived from the endothelium of the capillaries, proliferates and becomes more prominent." The evidence on which this conclusion was based was not produced.

Whether it is formed wholly by new fibrous tissue, wholly by old, or by both old and new, the perimedullary fibrous band remains, at least until 338 days, slightly broader in the female than in the male gland; hence Miller's (1927) statement that, after the *X* zone has disappeared, "the structure of the adrenal of the...female mouse is not different from the structure of the adult male gland" does not exclude this difference of degree.

3. *The time of complete involution*

When the *X* zone presents one of the various pictures of incomplete involution it is sometimes impossible to be certain whether cells with deeply staining nuclei adjoining the medulla are degenerate *X* zone cells or lipoid-free cells of the permanent cortex. A convenient rule would be to regard involution as complete when, in the absence of certainly recognisable *X* zone cells within the meshes of the perimedullary fibrous band, the inner edge of the perimedullary fibrous band is perfectly smooth. This rule would circumvent the difficulties due to the long persistence of small groups of *X* zone cells internal to the perimedullary fibrous band and to the appreciable variations in its width.

4. *Deanesly's "new cortical zone"*

Deanesly (1928) described the appearance of a "new cortical zone" after the disappearance of the *X* zone, based on the study of six female mice. The right gland was removed from each mouse four to seven weeks after the left gland. The glands from four of the mice are briefly described. The account of the "zona reticularis" of the left gland from mouse SCM. 11 corresponds exactly with the appearance of the inner part of the permanent cortex when free from lipoid. This "zona reticularis" was found to be larger in the right gland, and Deanesly stated that "the blood vessels and fibrous tissue next the medulla, however, appear to be extending into it, and it is probable that degeneration of the type found in the *X* zone is beginning." These glands were not illustrated; the evidence on which the conclusions were based was not produced; and the type of degeneration was not specified. The greater breadth of the "zona reticularis" may be due to decrease of cortical lipoid in the remaining gland after unilateral suprarenalectomy. This would be the simplest explanation of the appearances in the glands from mouse SCM. 4 also. Again, it was the right gland in which the "reticular zone" was well-marked, whereas in the left gland removed earlier "no definite inner zone" could be traced. It is stated that in the right gland "the fibrous reticular tissue round the

medulla has only penetrated the zone appreciably in one place." No evidence was adduced to support this statement. The site of what was thus assumed to be penetration seems to be illustrated in Deanesly's Fig. 6, where the low magnification does not allow details to be made out. It is significant that it occurs near one of the poles of the gland, and that, as stated in the corresponding legend, "the section lies to the outside of the median plane of the gland." This appearance, not rare in my own preparations, may result if the long axis of the gland is not parallel to the plane of section. The "zona reticularis" of the right gland of mouse SCM. 4 was "extensively vacuolated and hyperaemic." It was not illustrated. The "zona reticularis" of the right gland from mouse SCM. 15 "contains a number of degenerating cells." This gland is illustrated in Deanesly's Fig. 7, whose low magnification prevents appreciation of details. Paragraph (7) of the summary reads: "A new inner zone may arise later in the cortex; this though similar to the earlier one is distinguishable from it, but is also of a transitory character." No evidence was produced to show that it is transitory. The distinction between the two zones is, in my experience, simply that between X zone and permanent cortex which happens to be free from lipid. In a later communication it will be shown that sudanophil lipid occupies the whole or almost the whole permanent cortex while the X zone is present, in both sexes, and that when the X zone disappears, an increasingly broad zone round the medulla becomes lipid-free. This general tendency to decrease of lipid with age must be taken into account along with the effects of unilateral suprarenalectomy on cortical lipid in the remaining gland, a question reserved for detailed study. At present there is no convincing reason for regarding lipid-free permanent cortex round the medulla as a zone with a distinctive character like that of the X zone.

5. Identity of phase in each pair of glands

For experimental purposes it is useful to know whether in each of a pair of glands the X zone is in the same phase. Miller (1927) found that both glands from a single mouse were in the same phase. Deanesly (1928) confirmed Miller's observation. All the glands in my series were examined to check this, and without exception each pair of glands showed the X zone in the same condition. This does not mean that throughout a single gland the X zone had reached the same stage of involution. As shown above, the X zone may exhibit different degrees of involution in different parts of the same gland; in this event, however, the same stages are visible in the X zone of the other gland from the same mouse. The X zone is usually of the same width in both glands from the same animal.

SUMMARY

1. No evidence that the cytoplasm of the cells of the X zone of the mouse suprarenal is basophil was obtained.
2. It is shown that the female X zone may involute without fatty change.

3. Deanesly's (1928) observation that involution by fatty change appears to be the less frequent mode is confirmed.

4. It is suggested that the perimedullary fibrous band that succeeds the *X* zone may be formed by collapse of the *X* zone stroma.

5. Until 338 days at least, the perimedullary fibrous band is appreciably broader in the female than in the male gland.

6. The observations here discussed lend no support to Deanesly's (1928) view that a second transitory zone succeeds the *X* zone.

7. It is suggested that the appearances taken to indicate a new zone are identical with those of permanent cortex whence lipoid has disappeared.

8. The observations of Miller (1927) and Deanesly (1928) that the *X* zone is in the same phase in both glands from one mouse are confirmed.

9. When the *X* zone is in various stages of involution in one and the same gland, the same stages are visible in its fellow.

10. The *X* zone is usually of the same width in both glands from one mouse.

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EXPLANATION OF PLATE I

Fig. 1. Early stage of involution of male *X* zone. Upper half of figure shows cortex; lower half, medulla. Between permanent cortex and medulla, the *X* zone is visible as a band of cells about five cells broad, staining rather more darkly than the permanent cortex. The nuclei are more closely packed in the *X* zone than in the permanent cortex. Separating *X* zone from permanent cortex are a few fibrous tissue cells with elongated nuclei parallel to the gland capsule. The edge of the medulla is irregular, its cells interdigitating with those of the *X* zone. From a male aged 28 days. H. and E. after bichromate. $\times 200$.

Fig. 2. Adult male suprarenal. Upper left of figure shows permanent cortex; lower right, medulla, dark after bichromate. There is no trace of the *X* zone. The edge of the medulla is perfectly even, as in all adult glands. The thin band of cells immediately adjoining medulla is permanent cortex cells free from lipoid. They should not be mistaken for *X* zone cells. From a male aged 57 days. H. and E. $\times 200$.

Fig. 3. The broad dark zone crossing the figure from top right to bottom left is a healthy *X* zone. Top left shows permanent cortex, staining more lightly. Bottom right shows darkly stained cells of medulla, which interdigitate with the cells of the *X* zone. From a female aged 111 to 112 days. H. and E. after bichromate. $\times 200$.

Fig. 4. Another part of same gland as that shown in Fig. 3. Top left, cortex; bottom right, medulla, with the smooth border characteristic of maturity. Adjoining medulla, in the centre of the figure, is a cortical area containing many blood spaces. The *X* zone cells have disappeared. Involution is thus complete, but the shrinkage of the framework of the *X* zone that results in the formation of the perimedullary fibrous band has not yet occurred. H. and E. $\times 200$.

Photographs by H. C. Taylor.

REFERENCES

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Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.