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HISTOCHEMICAL OBSERVATIONS ON NODULAR PORTIONS IN ADRENAL CORTEX OF AGING RATS

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Nodular portions of the adrenal cortex in the aging rats were examined histochemically.

From the observations on lipid distribution and histological feature, the cortical nodules were classified into the following three types:

1) Type I consists of the compact cells containing no or scanty fine granules of lipid.

2) Type II consists of the large cells containing moderate amounts of small of middle sized granules of lipid.

3) Type III consists of the cells with vacuolated cytoplasm and abundant lipid droplets.

In the nodule of Type III, increased activity of secondary alcohol dehydrogenase was usually consistent. On the other hand, histochemical activities of the enzyme tended to more variable in Type I and Type II.

The possible relations of these nodules including few transitional forms were considered.

Many histopathological observations on the cortical nodules have been reported in the human adrenal glands[3,4,5,7,12,13,14]. However, their histogenesis, histochemical characteristics and functional significance have not been fully clarified. In the present study, histochemical investigations on the cortical nodules are demonstrated and the relationship between intracortical nodule and adenomatous hyperplasia will be also discussed.

MATERIALS AND METHODS

Thirty-three male Wistar rats aged 15–34 months, weighing 350 to 400 g, were used for this study. They were kept in 24°C with 60 per cent humidity for about one year and thereafter were maintained a room with a temperature of 5°C to 30°C. The systolic blood pressure of these rats were 100–120 mmHg.

After sacrifice, both adrenal glands were quickly removed and each adrenal was serially sectioned at 10 μ in a cryostat and stored at −25°C. Some sections were stained either with hematoxylin and eosin or Oil red 0 to examine the general lipids. Other sections were subjected to histochemical technique for the demonstration of steroid 3 β-ol dehydrogenase (3 β-ol DH) using dehydroepiandrosterone as the substrate[8], glucose-6-phosphate dehydrogenase (G-6-PDH) using glucose-6-phosphate sodium as the substrate[10] and secondary alcohol dehydrogenase (SADH) using isopropanol as the substrate[6].

Adrenal glands of other 15 rats were fixed in formol-calcium for 48 hours, and their frozen sections 10 μ in thickness, were used for the demonstration of non-specific alkaline phosphatase (Al-Pase)[9].

RESULTS

Forty-seven nodular portions were encountered in the both adrenal glands of 33 male rats examined.

Most nodules were localized to the zona fasciculata but some of them were found in the fasciculo-reticular zone. Although these nodules were usually solitary and unilateral, 9 of 33 rats showed multiple development in either adrenal gland. Five rats relealed bilateral nodular portions and 3 rats had the nodules more than two on the single adrenal.

The frequency of development of cortical nodule tended to increase correspondingly with the aging process.

The nodules were sharply demarcated from the surrounding cortex, especially by the demonstration of lipid distribution. Compression of the adjacent tissue by proliferating cortical elements was prominent but these areas were not encapsulated. Myelolipomatous metaplasia within nodular portions which has been reported in our other paper[12] was not seen in this study. In some cases, smaller nodules appeared to have fused to form the adenomatous nodule.

From the characteristics of quality and quantity of lipids and cellular elements, the cortical nodules of the aging rats were classified into the following three types.

1) Type I consists of the compact cells containing no or scanty fine granules

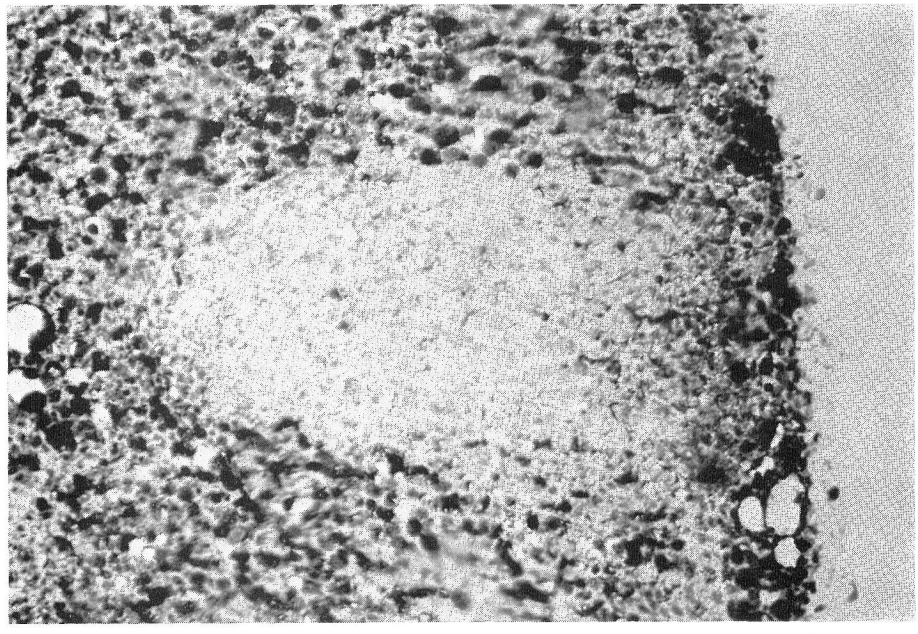


FIG. 1. Cortical nodule of Type I contains scanty fine granules of lipids. Oil red 0 stain. ×100

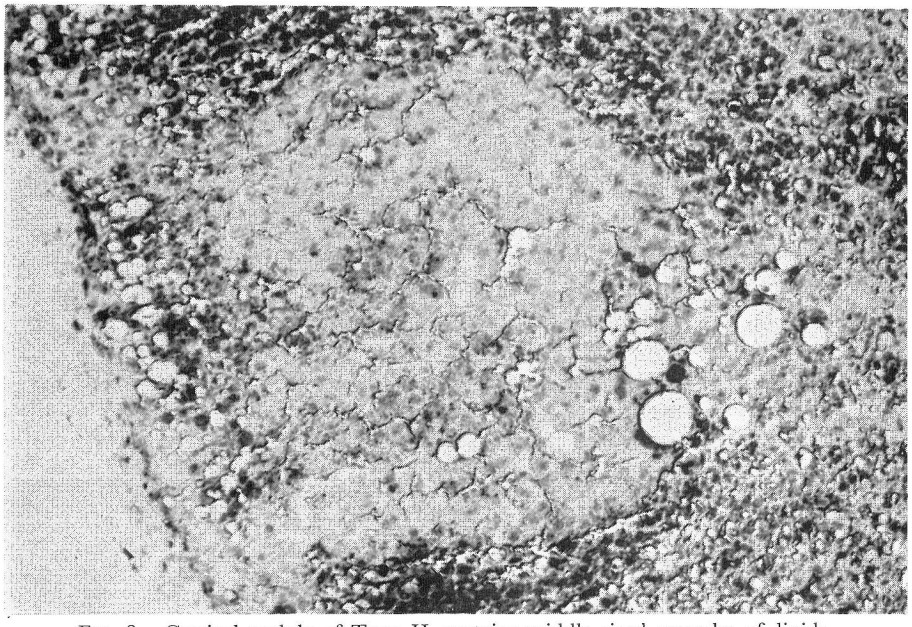


FIG. 2. Cortical nodule of Type II contains middle sized granules of lipids. Oil red 0 stain. ×100

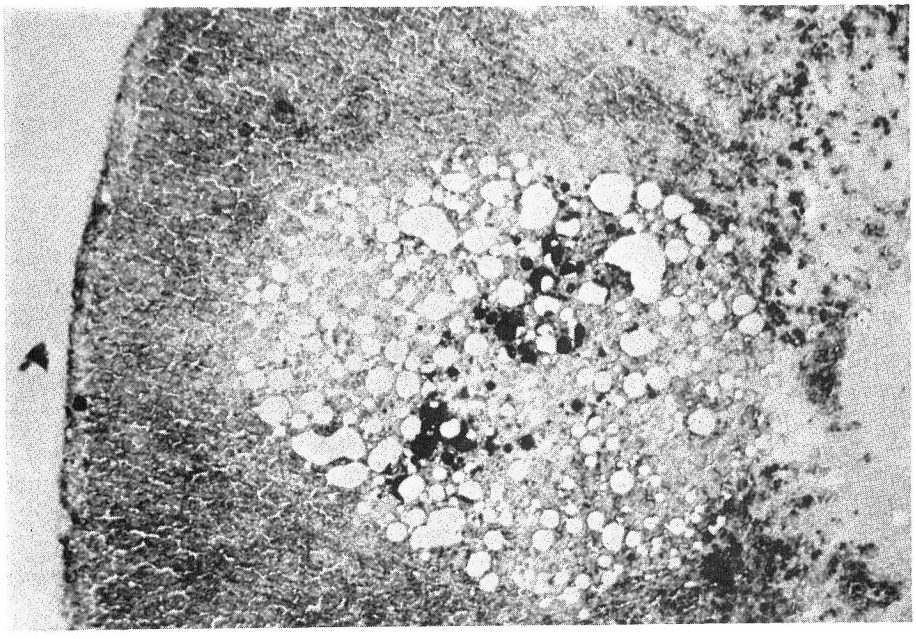


FIG. 3. Cortical nodule of Type III consists of the cell with vacuolated cytoplasm containing abundant lipid droplets. Oil red 0 stain. ×100

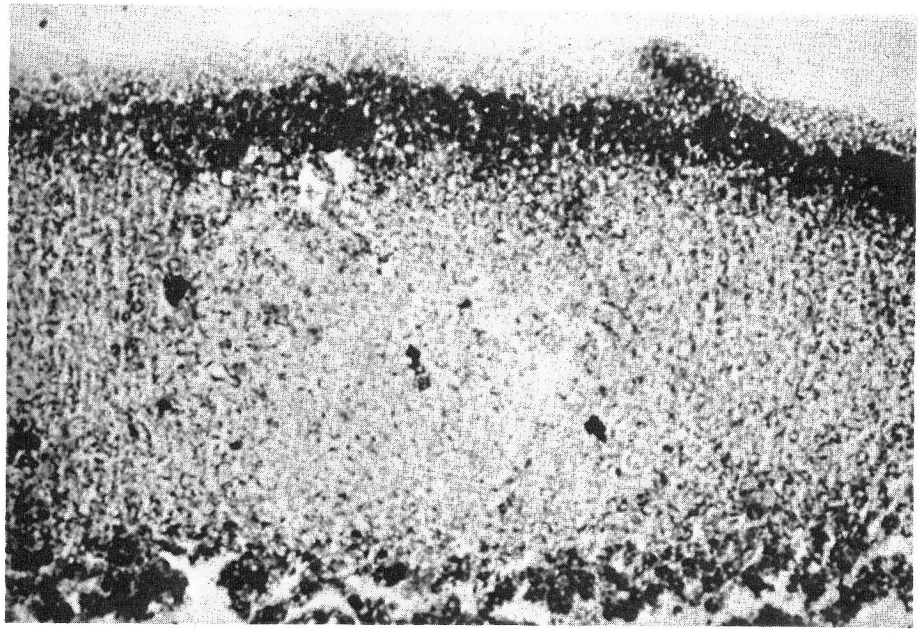


FIG. 4. Cortical nodule of Type I shows a decrease in 3 β-ol DH activity. $\times 60$

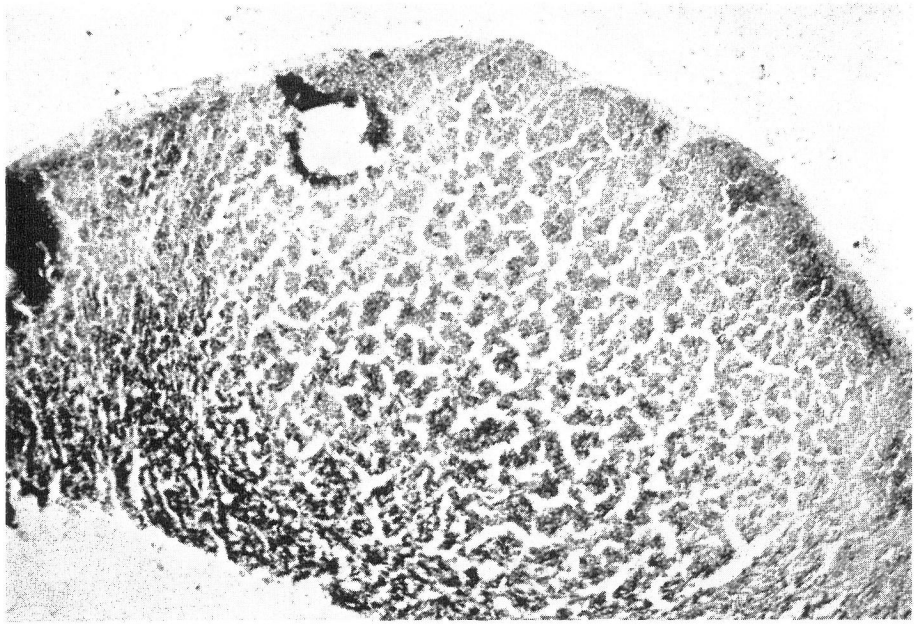


FIG. 5. Similar pattern of G-6-PDH activity to the surounding cortex is noted in the nodule of Type II. $\times 40$

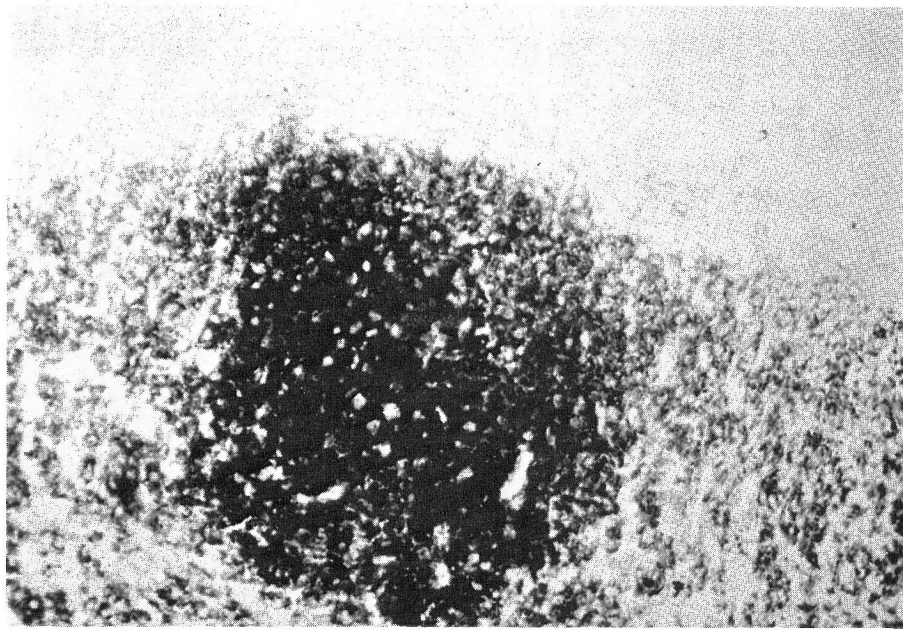


FIG. 6. Cortical nodule of Type III reveals strong activity of SADH. ×100

of lipid (Fig. 1).

2) Type II consists of large cells containing moderate concentration of small to middle sized granules of lipid (Fig. 2).

3) Type III consists of the cells with vacuolated cytoplasm and abundant lipid droplets (Fig. 3).

There were also few transitional or intermediate forms among these three types of nodule. That is,

4) Nodular portion consists of the compact cells containing small amounts of fine granules of lipid.

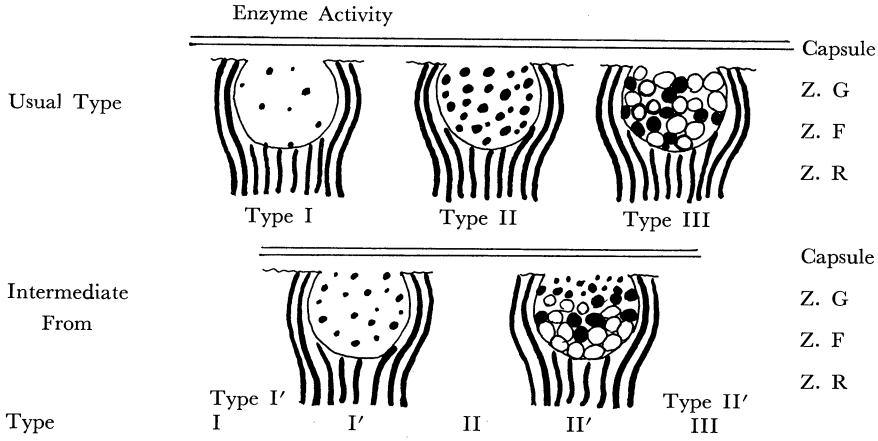
5) Nodular portion consists of the admixture of the cells with fine granules of lipid and the cells with vacuolated cytoplasm containing abundant lipid droplets.

Most nodule of Type I showed histochemically a marked increase in SADH activity, but there was no consistent tendency in the activity of G-6-PDH and 3 β-ol DH (Fig. 4). The nodule of Type II could not be distinguished from the surrounding cortex in G-6-PDH activity except one case (Fig. 5), and, on the other hand, SADH and 3 β-ol DH activities revealed some variability from nodule to nodule. Intensity of SADH activity was greater in the nodule of Type III (Fig. 6), while the activity of Al-Plase was decreased. The pattern of 3 β-ol DH and G-6-PDH activities similar to that in non-nodular areas of the cortex.

These histochemical results of cortical nodule are summerized in Figure 7.

COMMENT

In this study, nodular portions of the rat adrenal glands could be distinguished



Type	I	I′	II	II′	III
Numbers of Nodules	8	10	7	11	11
Enzyme Activity					
SADH	↑↑6, →2	↑↑7, →3	↑↑4, →2, ↓1	↑↑10	→10
3β-olDH	↗4, ↘4	↘8, →2	→5, ↗1, ↘1	→10	→10
G-6-PDH	↗6, →2	→8, ↗2	→6, ↗1	↗7, →3	→10
Al-Pase	↗3/3	→6/6	→6/6	→6/6	↑2/2

→Enzyme activity is similar to the surounding cortex.
↑ increased than the surounding cortex.
↗slightly increased.
↓ decreased.
↘slightly decreased.

FIG. 7. Classification of cortical nodules by lipid distribution and their enzyme histochemistry

from the surrounding cortex in terms of the lipid distribution and cellular component. Several enzyme activities also often differed from the adjacent non-nodular cortex.

Histological characteristics of nodules were closely related to the pattern of lipid distribution. With the exception of SADH activity, however, a feature of the enzyme activity in nodule was generally dissimilar to the lipid pattern. In the nodule of Type III, increased activity of SADH was usually consistent. On the contrary, other enzyme activities tended to be more variable from case to case and/or from nodule to nodule.

According to Hardonk, secondary alcohol dehydrogenase[6,11] seemed to be related to the dehydrogenase activity in the transformation from 20 α-22R-dihydroxycholesterol to pregnenolone, which does not have to occur in the same cells of steroid-producing tissue as the oxidation of the 3 β-ol group[1,8,17]. The

activity of this enzyme frequently highlighted in cortical nodule, paralleling the great intensity of lipid distribution observed with the Oil red 0 staining. The functional significance of such lipid accumulation in adrenal tissue is that secretory activity is rather depressed[13,14]. In other words, it tends to play a role in the storage of steroid precursors.

Such characteristics were particularly shown in the nodule of Type III. Therefore, this type could be interpreted as an inactive and storage phase of the cortical nodule. On the other hand, the enzyme activities in each nodule of Type I and Type II often varied. Moreover, both types of nodule contained considerably less lipid than the nodule of Type III. These observations led us to suggest that the nodules of Type I and Type II, particulary the latter, may have a more potentiality for steroid production and may reflect a greater capacity of the nodular cells to respond to various stimuli. The detail of relationship between ultrastructural alterations of the cell organelles and their functional significance in cortical nodules will be reported elsewhere[12].

In this series of experiments, we have found the transitional forms of nodule among these three types. From the cellular element in the nodule of transitional form, there is some possibility of transformation from Type I to Type II, or Type I to Type III, or Type II to Type III. In addition, we have previously observed[12] that large adenomatous nodules were predominantly composed of Type I or Type II, and that they sometimes were intermingled with transitional form to Type III. These relations support the probability of a fundamental link between intracortical nodule and adenomatous mass. This conception is comparable with the hypothesis by Cohen[3] in a study of surgically resected glands with Cushing's syndrome.

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