The development of the permanent adrenal cortex of the fetus in the last quarter of gestation was histometrically examined. The volume of the permanent cortex did not increase in proportion to the total adrenal or body weight, but its growth kept pace with increase of adrenal surface area. The thickness of the permanent cortex did not essentially increase with the progress of fetal months.

The permanent cortex was composed of columnar lobules demarcated by reticulin fibers. The diameter of the lobules became smaller with advancing fetal months, on account of subdivision of the lobules by invading reticulin fibers. The maturation of the permanent cortex in late pregnancy consisted consequently in subdivision of the lobules but not in an increase in the height of the lobules.

The difference in the behavior of the permanent cortex between mature and premature newborns was that in the course of normal development. No deviation from the normal development was found in the permanent adrenal cortex of the premature.

As maturity of fetuses correlates well with body weight at birth, premature infants are generally expected to have smaller adrenals than mature ones, which would explain their inadequate adaptability to postnatal surroundings. This concept presupposes a correlation between the growth of the fetal adrenal glands and the development of hormonal activities. Gelfman1 stated that there was no definite relationship between the size of the adrenal and body weight. However, Dhom et al.2 could discriminate the mature and immature by an index of adrenal weight to body weight. It is still to examine how the adrenal weight is correlated with the function.

If corticosteroids play an important role in adrenal adaptation, the adrenal cortex deserves special attention. The fetal adrenals have two cortical layers, fetal and permanent. The fetal zone with its large volume occupies a greater part of the gland. Dhom et al.2 confirmed by micrometric methods on histological sections that the fetal zone remarkably increased in its area with progress of gestation. Simultaneously, the nuclear volume of the constituent cells was found increased. From the fact that an increment in corticosteroid content of the fetal

Received for publication, September 25, 1969.
adrenals was more remarkable in the later gestation than the gain in the weight of the glands, they concluded that the nuclear volume would indicate secretory function. Their view was supported by later works demonstrating that the nuclear volume in the glomerulosa decreased after administration of aldosterone and that in the fasciculata increased in the ACTH-treated patients and conversely decreased in the cortisone-treated. These results of karyometry seem to indicate the importance of a quantitative morphological analysis.

However, there remain several problems about karyometry. Even if the nuclear volume is parallel to cellular function, it is interpreted only as an expression of transient cell function and cannot demonstrate whether or not the suprarenal gland retains its normal development or is exhibiting enhanced activity in response to environmental changes.

According to the widely accepted opinion, the cells in the permanent zone migrate into the fetal zone, which is subject to degeneration shortly after birth. Therefore, the present investigation may be confined to the permanent zone, which plays a major part in postnatal development. Rotter demonstrated a remodeling of the permanent zone with progress of the gestational month. The cells in the permanent zone are covered with arch-formed caps of fibrous connective tissue, from which collagenous fibers extend vertically downwards and separate the fasciculata. The lobuli called the zona fasciculo-arciformis by Rotter become splitted into thinner cellular strings by argyrophil fibers descending from the capsule as the term approaches. By the strings the zona glomerulosa is defined after delivery.

However, his view is not generally accepted. The permanent zone is well differentiated in some of the immature infants, while incomplete splitting is observable in the mature. In many cases the layer is probably of intermediate differentiation, and the grade of differentiation is not always recognizable by usual histological examinations.

In serial horizontal sections of the fetal adrenals the cells of the zona fasciculo-arciformis were found encircled by descending fibers with capillary networks and arranged in columnar lobules, which were bundled and packed closely in the permanent zone. Consequently, we regard the arciform lobule as a morphological unit of the zone and apply our histometrical methods to analyze structural changes in the adrenal cortex of the fetuses.

**Materials and Methods**

The adrenals of still- and new-born babies within 7 postnatal days were obtained from 36 autopsy cases comprising 9 in the eighth fetal month, 9 in the ninth and 18 in the full term. Tissue specimens of exact transverse adrenal section were fixed in Zenker-formalin and embedded in paraffin. Histological sections of 4 μ in thickness were stained with hematoxylin-eosin and modified Gomori’s silver stain. The specimens were histologically examined and absence of advanced involution and other serious pathological findings was assessed. Serial horizontal histological sections covering the entire permanent zone were prepared from pertinent cases of each gestational month.
1) **The weight of adrenal parenchyma and total surface area of the adrenals**

The adrenals are usually weighed together with different amounts of pericapsular fat tissues. Therefore, the net weight of the adrenal parenchyma must be determined at first in the present study. Histological slides were magnified and projected for camera lucida drawings. The surface area of the total cross section of the specimen and that of the adrenal parenchyma were estimated planimetrically, while the length of capsular section was determined by attaching thin cotton threads on the depicted line. If we assume that the ratio of the adrenal parenchyma to the total cross section is constant in all sections of the both adrenal glands and that the specific gravity is uniformly equal to 1.0, the product of the ratio and the gross weight of adrenals will give the net weight of the adrenal parenchyma.

Simultaneously, the total surface area of both adrenals $S$ can be calculated by:

$$ S = L V_a / A, $$

where $V_a$ is the volume of both the glands, $A$ is the total cross-sectional area on the histological slide and $L$ is the length of the capsular line.

2) **The mean diameter, height and volume of arciformed lobules in the permanent zone**

Reconstruction of the lobules in the zona fasciculo-arciformis by means of silver-stained serial sections of several cases revealed that the radius of curvature of the arch was unexpectedly large in comparison with the lobular height, and that the arciformed lobule itself could be regarded as a simple column. It was also established that the diameter of these columns showed little variance in the full-term infants and the eighth month of gestation and the deviation could be disregarded.

On the histological slide of cross section the width of an arciformed column was determined by the scale of an ocular micrometer. One hundred lobuli were thus successively determined for diameter on the same histological slide. Based on the assumption above, a product of the mean height of columns, which was equal to the mean depth of the permanent zone, and the total surface area of adrenals gives the volume of the permanent zone. In addition, the average volume of lobuli can be calculated from the formula of columnar volume.

3) **The total number of lobuli in the zona fasciculo-arciformis**

If the zona glomerulosa foetalis is made of columnar lobules of the same diameter piled as in Fig. I, the total number of the lobules in both adrenals $N_A$ would be calculated by:

$$ N_A = 2 \sqrt{3} \frac{L V_a / 3 A}{D^3}, $$

where $V_a$ is the volume of the adrenals.

4) **The number of incomplete columns in the upper part of each arciformed lobule**

In a transverse section arciformed lobules may be subdivided by a few thin fibers descending from the capsule. According to our observations by means of reconstruction of horizontal sections, these argyrophil fibers from only incomplete septa in a lobule. However, they contain capillaries and are expected to give rise to new lobules. These intralobular septa were so tortuous that a precise quantitative estimation is hardly possible. The number of these incomplete columns in each lobule were counted on transverse sections.

5) **Orthogonal mean square regression**

In examining the regression of quantities determined in the present histometrical study.
the principle of orthogonal mean square regression was applied. The regression coefficient is obtained by:

\[ b = \frac{1}{2S_{xy}} \left[ (S_{y^2} - S_{x^2})^2 + \sqrt{(S_{y^2} - S_{x^2}^2 + 4(S_{x y})^2)} \right], \]

where \( S_{x^2} \) and \( S_{y^2} \) are the sums of squares of two variables and \( S_{xy} \), the sum of products.

RESULTS

1) Gross and net weight of the adrenals

The combined weight of both adrenals at autopsy was plotted on the abscissa against the net weight of the parenchyma on the ordinate of a double-logarithmic

\[ W_{ap}(g) \]

\[ W_a(g) \]

\[ W_{ap}(g) \]

\[ W_a(g) \]

Fig. 2. Gross weight and net weight of the adrenals. \( W_a \): gross weight. \( W_{ap} \): net weight of the adrenal parenchyma. The regression coefficient of 1.02 indicates that net weight increases in proportion with gross weight.

2) Body weight at birth and gross adrenal weight at autopsy

Body weight at birth was plotted along the horizontal axis and adrenal weight along the vertical one (Fig. 3). The correlation coefficient of 0.70 at degrees of freedom of 28 indicated a statistically significant correlation between the two variables, but in view of the limited range of body weight in the present study, a further investigation seems still necessary. Relative weight of adrenals to body weight was also calculated, but there were only two cases in which the ratio surpassed the limit of 1: 800, an index of immaturity according to Dohm et al.2

3) Relations of adrenal weight to the mean diameter, height, volume and the number of arciformed lobules, and the volume of the permanent zone

Adrenal weight was plotted on the abscissa and one of the other variables on the ordinate of a double-logarithmic system (Figs. 4 and 5). The coefficients of
There is a significant correlation between the adrenal weight and the volume of the permanent zone and the regression coefficient is 0.79, which is, however, not significantly different from 2/3, but significantly lower than 1. The regression coefficient was 1.23 between the adrenal weight and the number of lobules, or the number of lobules was found to increase with the increase of adrenal weight. The mean diameter, height and volume of the lobule also demonstrated negative correlations.

4) Relations of body weight at birth to the mean diameter, height, volume and number of the arciformed lobule, and volume of the permanent zone

The body weight at birth was plotted on the horizontal axis of a double-logarithmic system. The correlation coefficient and the orthogonal mean square regression coefficients are shown in Table 1 and Figs. 6 and 7. The birth weight
Fig. 5. Adrenal weight and volume of the outer zone. \( W_a \): combined adrenal weight. \( V \): total volume of the permanent cortex. The regression coefficient is 0.79, its confidence interval being 0.67 to 0.91 at 5\% level.

Table 1. Structural parameters of the cortical lobule and adrenal or body weight

<table>
<thead>
<tr>
<th></th>
<th>Adrenal weight ( (g) )</th>
<th>Body weight at birth ( (g) )</th>
<th>Volume of the outer zone ( (cm^3) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r )</td>
<td>-0.50</td>
<td>-0.83</td>
<td>-0.43</td>
</tr>
<tr>
<td>( b )</td>
<td>-0.12</td>
<td>-0.10</td>
<td>-0.41</td>
</tr>
<tr>
<td>( n )</td>
<td>30</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>( r )</td>
<td>-0.57</td>
<td>-0.71</td>
<td>2.37</td>
</tr>
<tr>
<td>( b )</td>
<td>-0.41</td>
<td>-1.03</td>
<td></td>
</tr>
<tr>
<td>( n )</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
</tbody>
</table>

\( n \): number of pairs. \( r \): correlation coefficient. \( b \): orthogonal mean square regression coefficient.

and the mean diameter of arciformed lobules are highly correlated with each other with a regression coefficient of \(-0.38\). Between the body weight and volume of the lobule a negative correlation was also established. No correlation was found to the mean height of lobules.

5) Number of incomplete columns in the upper part of each lobule

Through one hundred lobules examined there was no definite relation between the arithmetic mean of the number of columns and the body weight at birth. But,
Fig. 6. Body weight at birth and mean diameter of arciformed lobules. W: body weight at birth. D: mean diameter of the arciformed lobules. The regression coefficient is $-0.38$ with a confidence interval of $-0.30$ to $-0.46$ at 5% level.

as shown in Figs. 8 and 9, premature infants displayed significantly larger deviations than the mature.

**COMMENTS**

According to Dhom et al.² and Dhom,⁷ there is only insignificant increase in the volume of the outer cortex in the fetal period in comparison with pronounced gain in body weight, and there is no difference in the volume of the outer cortex between the mature and premature infants. We have also obtained essentially the same result. There is no correlation between the volume of the outer cortex and birth weight. The result suggests that the volume of the outer cortex does not increase with fetal growth. It appears as if there were no development of the outer cortex with advancing fetal months in late pregnancy.

On a double-logarithmic co-ordinate system the regression coefficient of the volume of the outer cortex upon the total adrenal weight is significantly lower than 1, and it may be regarded practically as being close to $2/3$. This means that the growth of the outer cortex does not keep pace with the increase of the adrenal weight, but it is proportional to the increase of surface area of the organ. The thickness of the outer cortex or the height of the lobule does not significantly increase with the advance of fetal months of late pregnancy.

On the other hand, negative correlation was found between birth weight or adrenal weight and lobular diameter. The result indicates that the maturation
of the outer adrenal cortex proceeds in the form of subdivision of the cortical lobules. Prevalent incomplete splitting of the lobules by invading reticulin fibers in premature infants is a histological proof of progressive lobular subdivision in late pregnancy. Our results are in agreement with the histological observation of Rotter. The subdivision of the lobules increased the contact of cortical cells with interstitial capillaries and may be regarded as anatomical expression of functional maturation of the adrenal cortex.

Kloos and Staemmler regarded adrenocortical insufficiency as one of the causes of death in premature infants. Histologically demonstrable immaturity of the cortex may well account for a functional insufficiency. However, it must be pointed out at the same time that, so far as lobular structure is concerned, the permanent cortex of the premature represents merely a premature stage of normal adrenocortical development. There is no qualitative difference.
Fig. 8. Distribution of the number of incomplete columns in a lobule in the 9th gestational month. n: the number of incomplete columns in a lobule. Body weight: 1,600 g.

Fig. 9. Distribution of the number of incomplete columns in a lobule at full term. Body weight: 2,840 g.

References