THE DEVELOPMENT OF THE SUPRARENAL GLANDS

IN THE GUINEA-PIG (CAVIA COBAYA)

by

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INTRODUCTION

The purpose of this study of the development of the suprarenal glands in the guinea-pig has been to ascertain the period in foetal life at which these glands first make their appearance, the ultimate source from which they are derived, and their differentiation.

REVIEW OF THE LITERATURE

One of the early workers on the adrenals was Biedl (1897), who found in the blood from the adrenal vein of the rabbit a considerable number of highly refractive granules which he thought to be the active principle of the secretory substance from the medulla.

Elliott and Tuckett (1906) found that the medulla in the guinea-pig is small in bulk; that it attains its full development by the end of the first month of post-natal life; that it is the storehouse of adrenalin; and that the cortex continues to grow in close parallelism with the animal's weight and attains great size.

Elliott and Armour (1911) studied human fetuses and post-natal stages of development, and noted a very large adrenal gland in relation to the kidneys. This they asserted is due to a peculiar hypertrophy of the cortex in foetal life. At birth the glands are composed of a thin core of medulla, a narrow rim of cells from which is derived the adult cortex, and a large foetal mass of cortical cells, which degenerate. At the third month after birth the absolute weight of the gland was less than at birth, and by the end of the first year the foetal cortex had vanished, and its place had been taken by the true cortex.

Kuntz (1912) noted in the Loggerhead turtle during the 19th day of foetal life that there is a ventral migration of the sympatho-chromaffin cells from the prevertebral sympathetic plexuses as far as the ventral level of the aorta. He found, also, that the cortical cells arise during the eighth day of incubation as buds on either side of the aorta, from the peritoneal epithelium just lateral to the root of the mesentery and approximately at the middle level of the mesonephros.

Hays (1914) states that the chromaffin substance in the chick begins its ventral migration from the anlagen of the prevertebral sympathetic plexuses at the 120th hour

(five days) and that it is composed of cells which are larger than those of the cortex and take a deeper stain. The cortical cells make their appearance during the 96th hour of incubation as thickenings of the peritoneal epithelium ventral and mesial to mesonephros, ventral to the abdominal aorta and dorsal to the hind gut, and continues development until the 168th hour, at which time connective tissue fibres begin to collect around the gland. He says that there is no sharp division into a cortex and medulla in birds.

Wieman (1920) says that the adrenals in human embryos of nine millimeters and 12 millimeters are distinctly marked off from the surrounding tissues and that the median side of each gland is in close contact with the prolongation of the ramus communicans and masses of ganglion cells, some of which seemed to be migrating along the distal fibres of the ramus to form the coeliac and visceral plexuses.

Weymann (1922), in pig embryos of 40 to 45 millimeters, found that the medullary cells arise from the coeliac sympathetic plexuses; that the chromaffin granules are beginning to develop in them; and that the cortical cells, into the interstices of which the chromaffin cells migrate, originate in the coelomic epithelium.

Keene and Hewer (1927) saw in human embryos of five millimeters to full term that the sympathetic anlage made its appearance at five millimeters; the cortical anlage is evident at 10 millimeters; sympatho-chromaffin cell migration begins at 12 millimeters and becomes most active between the 12th and 22nd week; and the foetal cortex degenerates during the last 10 weeks of foetal life. The cells composing the medulla consist of two types--one of small cells with darkly staining nuclei and little protoplasm and the other of cells larger than neuroblasts, and which have vesicular nuclei but smaller than cells of the foetal cortex.

Hill (1930), working with Primates (monkey, marmoset, and lemur) Carnivora and Ungulata, observed that the two latter orders have no marked enlargement of the adrenals at birth; that the adult glands are smooth; that the cortico-medullary boundary is indefinite in the young animals, due to the absorption of the intra-medullary islands of the cortex, or a down-growth of the same, and that the cellular structure of the cortex in the young is similar to that of the foetal cortex of Primates.

Pankratz (1931) worked out the unfoldment of the adrenals in the albino rat. He stated that the first appearance of these glands is during the 13th day of foetal life

as thickenings on the coelomic epithelium medial to the cephalic portion of the mesonephric fold, and that the cells comprising these are larger than those from whence they came, have vesicular nuclei, and migrate dorsally into the mesenchyme. At 16 days, the ectodermal components of the glands begin their ventral migration, as small darkly staining cells from the sympathetic ganglia toward the cortex, and later enter the vascular spaces at the first instance of motility. This migration continues until birth.

Hoerr (1931) found dark and light cells of the zona reticular, which he maintained degenerates, and are replaced by those from the area between the glomerular and fasciculate zones.

MATERIALS AND METHODS

Both serial sections and gross anatomical dissections were studied in the preparation for this thesis. Some of the material for the sections was fixed in Bouin's fluid and some in Muller's fluid. The stains used were borax carmine and Heidenhain's iron-alum haematoxylin. All of the material for sections was embedded in paraffin and mounted in balsam.

The dissections were made on both embryos and adults and consisted of explorations in the region of the adrenals

and of excisions and removal of the glands in order that a
comparative study might be made of the size, shape, loca-
tion, and cytological structure of these organs.

The embryos studied ranged from 14 to 31 days, in-
clusive. The adrenals from the adults were removed from
pregnant females whose embryos were also incorporated in
our study. In addition, adrenals were removed from ani-
mals of one-day post-natal life in order that a comparison
might be made at this stage, with those glands preceding
partuition, as well as those directly succeeding partui-
tion.

OBSERVATIONS

Position, Size, and Structure of the Adrenals

We approached the problem of the development of the
adrenals in the guinea-pig by first ascertaining the posi-
tion of these glands in very young embryos of 16 and 21
millimeters (25 and 31 days), respectively. They were
found to lie ventro-mesial and cephalad to the developing
metanephros, which position they maintain to and through
adult life. Even earlier--from the 22nd day--when the
gland begins to form, to its macroscopic size at about 25
days, the adrenal was seen, as in its adult position, ven-

tral, mesial, and cephalad to the permanent kidney. Even
though of only six days difference in ages, the adrenals
were much enlarged in the older of the two specimens--the
kidney measuring 2.5 millimeters and the adrenal gland 1.75
millimeters. The mesonephros appeared quite visible in the
16-millimeter stage, while in the 21-millimeter development
no mesonephros was seen.

A study of the cells at these stages of development re-
vealed that they are not circumscribed in definite areas,
as the medulla and cortex of later differentiation, but con-
sist of densely stained nuclei, the chromaffin bodies which
are well scattered throughout a syncytium of more lightly
stained nuclei. These chromaffin cells or bodies appear
uniform in shape, while the lighter stained cortical nuclei
are polyhedral in form and lie in a syncytium. This syncy-
tium arranges itself into cords, more or less simulating
liver tissue, but lacks the highly vascular appearance of
the latter. Mitotic figures were rare.

The Cortical Anlage

At 22 days of embryonic life, there is a thickening
of the coelomic mesothelium on the medial aspect of the
cephalic third of the mesonephros. This thickening con-
sists of nuclei somewhat larger than those of the surround-

ing syncytial area and of the contiguous mesothelium. These nuclei, arranged four layers in depth, are uniformly elliptical and contain from two to three nucleoli and fine granular chromatin particles (figure 1).

At this period of development these nuclei (for no cell walls can be seen at this stage) are forming the cortical anlagen of the adrenal glands by a migration dorsomedially into the mesoderm lying between the Wolffian ridge ventro-laterally and the aorta dorso-medially (figure 1).

From the 22nd day to the 27th this movement of specialized mesothelial nuclei was seen migrating between the smaller and more numerous polyhedral syncytial nuclei to the position in the mesenchyme, which is to become the permanent location of the adult glands. These mesothelial nuclei are the potential cortical anlagen of the adult adrenal glands.

The vascular nature of the adrenals is seen at this stage of development to be confined almost exclusively to the area immediately surrounding the primitive central vein.

The Medullary Anlage

Masses of deeply staining cells (the so-called chromidial substance, Nissl substance, tigroid bodies, chromophile or chromaffin substance) migrate from the prevertebral

sympathetic plexuses almost synchronously with that of the forming cortical anlage. There appears to be a great similarity between the pure sympathoblasts and the chromaffinoblasts which arise in common from the sympatho-chromaffin tissue, and it is only after the latter have migrated into the cortical anlage that they reach their ultimate differentiation into true chromaffin cells (figure 2). The chromaffinoblasts are larger than the sympathoblasts and do not stain as intensely as the latter. These darkly staining ectodermal constituents characteristically arrange themselves into circular or concentric formations known as 'rosettes,' which were seen in many of the sections studied (figure 2).

The chromaffin cells in the early period are somewhat larger than the cortical nuclei and are pushing into the cortex from its medial side and separating the cords of cortical cells into smaller units. The nuclei are finely granular, polyhedral in shape, with several nucleoli and have distinct nuclear membranes, but no cell walls were identified. In the 'rosette' formation a condensation of the syncytial mesenchyme was clearly seen. This gave to the masses of chromaffin cells a ring-like appearance (figure 2).

The aortic bodies which lie medially and in juxtaposition to the adrenals and which are derived from ectoderm send cords of chromaffin cells laterally into the forming cortex concurrently with that of the ventro-lateral migration from the sympathetic ganglia (figure 3). We interpret this to mean that the cells which comprise the aortic bodies, having come from the sympathetic ganglia, supply the medullary portion of the adrenals with chromaffin cells in part.

Further, in other sections of embryos, from 23 to 26 days, numerous cell migrations were noted passing ventro-laterally from the sympathetic ganglia to the adrenal anlagen. Hence, this migration from the sympathetic ganglia of cells which constitute part or all of the aortic bodies and the medullary portion of the adrenals appears to indicate a common source of origin for these organs (figures 3 and 4).

The Adrenal Gland at One-Day Post-Natal Life

Transverse sections of the adrenals at this period of development reveal glands which are triangular in outline and which have their two component parts well defined and in complete separation from one another (figure 6).

The medulla, too, is triangular in outline and is com-
posed of rather large masses of deeply staining chromaffin
bodies which completely obscure the nuclei of the cells
and which are predominantly the most characteristic con-
stituents of the medulla (figure 6). These bodies are
thought by physiologists to be the secretory primordium of
the substance known as adrenalin or epinephrin. This re-
gion in the adrenal is greatly vascular. The cells sur-
round a central vein into which the sinusoids converge to
empty their blood-laden adrenalin substance, and surround-
ing this is the cortical zona reticulata with its vascular
arterial system which brings in new blood to the gland
(figure 6).

The cortex at this stage of development has arranged
itself into three rather distinct areas or zones, namely,
the zona glomerulata at the periphery of the adrenal, the
zona fasciculata next to the glomerular zone, and most cen-
tral, the zona reticulata immediately surrounding the medul-
la. A connective tissue capsule covers the entire gland
(figure 7).

Characteristic conditions of the cortical cells are
their abundance of fat globules which occupy most of the
cytoplasm in the outer third of this region, and the pig-
ment granules in these cells, especially in the region ad-

jacent to the medulla. The embryonic gland contains no
cortical pigment and the fat is uniformly distributed
throughout the cortex, but at one-day post-natal life the
cellular inclusions as described become evident.

The reticular zone contains both light and dark cells.
This condition, no doubt, is due to the disintegration of
some of these cells followed by a subsequent replacement
from the fascicular zone, immediately in juxtaposition to
it. The reticular zone is quite free from fat, contains
many pigment granules, and occasionally some medullary
cells which may have wandered in among the light and dark
cells of this area (figure 7).

The cells of the fascicular zone are smaller than
those of the zona reticularis; arrange themselves into
chains; contain small deeply stained nuclei; have numerous
fat globules and a finely granular cytoplasm (figure 7).
Sinusoids occupy the interstices between the fascicles of
this zone.

Many inclusions of fat droplets fill the cells of the
zona glomerulosa. These cells are smaller than those of
the other two zones and are compressed against one another,
thereby reducing to a minimum the intercellular spaces so
characteristic of the more centrally located zones. Imme-

diately surrounding the zona glomerulosa is the connective tissue capsule (figure 7).

The Adrenal Gland of the Adult

A gross and a microscopic study of the suprarenal glands of the adult was made. These glands were taken from the mothers of the embryos which were used in our study.

Microscopically there was little, if any, difference in the cellular structure from that found in the adrenal of a one-day post-natal life guinea-pig. But the cortex had increased in size, and both it and the medulla had changed from a triangular to an elliptical shape. The vascularity of the medulla had increased, perhaps due to the effects of gestation upon the gland.

The length of the adult glands was approximately one centimeter; from side to side, three millimeters; and dorso-ventrally, six millimeters.

DISCUSSION

There are two general theories concerning the origin of the cortical and medullary substances, namely, the homogeneous and heterogeneous concepts. The former theory contends that both components of the adrenals are derived from

the same source while the latter theory upholds that the two constituents originate from two distinct and separate sources.

Some of the contenders of the homogeneous origin were Gottschau (1883), who asserted that the adrenal elements came from the mesenchyme; Balfour (1878), that they arose from the sympathetic ganglia; and Leydig (1853), that the source of origin was from the sympathetic nervous system.

The heterogeneous supporters are the more numerous. Poll (1906), Kuntz (1912), Hays (1914), Elliott and Tuckett (1906), Elliott and Armour (1911), Wieman (1920), Weymann (1922), Keene and Hewer (1927), Hill (1930), and Pankratz (1931) all assert that evidence favors the theory that the cortical substance is derived from the mesodermal epithelium and that the chromaffin substance develops from cells which break away from the anlage of the peripheral part of the sympathetic nervous system.

We have found in the guinea-pig that the cortex comes from the mesothelium on the medial side of the mesonephros and the medulla from the sympathetic ganglia and the aortic bodies. We are in accord with the theory of the double origin of these glands as upheld by the exponents of the heterogeneous concept, but we have not found any reference to the close relationship existing between the aortic bod-

ies and the suprarenal glands.

Of the number of papers which have been published on the development of the suprarenals we have found only two which have used the guinea-pig as a subject for investigation.

Elliott and Tuckett (1906) made a gross study of the adrenals of the guinea-pig by measuring the mass of them in transverse sections and thus they calculated the total area of the glands. They also found that the medulla was small in bulk; that it attains its full development by the end of the first month of post-natal life; and that the cortex continues to grow in close parallelism with the animal's weight and that it attains great size. They did not state, however, the period at which the cortex and medulla make their appearance, the source from which they are derived, and their differentiation. Nor did they mention the aortic bodies in connection with their studies.

Hoerr (1931) described light and dark cells of the zona reticulata in the guinea-pig and stated that they degenerate and are replaced by the cells from the area between the glomerular and fasciculate zones. He did not give the origin of the cortex, the medulla, nor the chromaffin cells. We also found these dark and light cells as described by Hoerr (1931).

It seems that the time of migration of the chromaffin
cells varies in different animals. Kuntz (1912) states
that in the turtle they migrate during the 19th day; Hays
(1914), in the chick, the fifth day; Keene and Hewer (1927),
in human embryos, the 12th through the 22nd week; and Pank-
ratz (1931), in the albino rat, the 16th day of foetal life.
Our investigation showed masses of darkly staining cells mi-
grating from their source of origin at the 22nd day to the
already forming cortical anlage, which we interpreted as
chromaffin cells.

Likewise, the period of the cortical formation varies
in different animals. Kuntz (1912) found in the turtle
that the cortical cells arise from the peritoneal epithe-
lium during the eighth day; Hays (1914), in the chick, the
fourth day; Keene and Hewer (1927), in human embryos, at 10
millimeters; and Pankratz (1931), in the albino rat, the
13th day of embryonic life. But in the guinea-pig we found
that the cortical cells are forming the anlagen of the adre-
nals at the 22nd day.

SUMMARY AND CONCLUSIONS

1. The cortical anlage makes its appearance at the 22nd day
   of foetal life and arises by a migration from the coelo-
   mic mesothelium on the medial aspect of the cephalic

third of the mesonephros.

2. The medullary portion consists of deeply staining cells
   known as chromaffin or chromaphile bodies.

3. These chromaffin cells are derived from the prevertebral
   sympathetic ganglia and the aortic bodies and begin their
   migration from their source to the already forming cor-
   tical anlage almost synchronously with that of the latter.

4. We conclude from this study and investigation that the
   adrenal glands in the guinea-pig have a heterogeneous
   origin--the medullary portion comes from the ectoderm,
   the cortical constituent from the mesoderm; that the
   time of origin is different from that found in other an-
   imals; and that the aortic bodies supplement the contri-
   bution made by the sympathetic ganglia.

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PLATE I

A microprojector and camera lucida were used in preparing the outline of the drawings.

Fig. 1. The cortical anlage at 22 days (X 19 o. i.). CN, cortical nuclei; CS, cortical syncytium; PCC, potential cortical cells; WD, Wolffian duct; CA, cortical anlage; GR, genital ridge.

Fig. 2. Rosette of chromaffin cells in adrenal at 24 days (X 19 o. i.). CC, chromaffin cells; CB, chromaffin bodies; S, syncytium.

Fig. 3. Cord of chromaffin cells migrating from aortic body to adrenal gland at 26 days (X 160). M, mesonephros; AG, adrenal gland; AB, aortic body; A, aorta; WD, Wolffian duct; GR, genital ridge.

Fig. 4. Cross section of guinea-pig embryo at 26 days (X 80). AB, aortic body; M, mesonephros; WD, Wolffian duct; SG, sympathetic ganglion; A, adrenal; H, heart; L, liver.

Fig. 5. Section through region of developing adrenal at 27 days (X 160). MES, mesonephros; WD, Wolffian duct;

MET, metanephros; AO, aorta; AB, aortic body;

A, adrenal; GR, genital ridge; L, liver.

Fig. 6. The adrenal at one-day post-natal life (X 80).

CV, central vein; M, medulla; C, cortex.

Fig. 7. Section through adrenal at one-day post-natal life

(X 19 o. i.). M, medulla; RZ, reticulate zone;

FZ, fasciculate zone; GZ, glomerulate zone; C,

capsule.