The Endocrine System

Morphology and physiology correlated during development

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SINCE it has been established that the endocrine glands are essential for growth, an understanding of the morphological and physiological activities of these glands during the process of development of the embryo becomes important. The endocrine glands compose a system which functions as a regulator in maintaining a balance of the physiological processes of living protoplasm. At present there is little information regarding the exact time in embryological development when the hormones of the various endocrine glands become available for use by the organism. Therefore, it is the purpose of this paper to correlate the morphological and known physiological activities of some of the developing endocrine glands of the chick embryo.

With regard to the chick embryo, Sun¹ has published an account which includes a morphological study of the thyroid, parathyroid, adrenal, and thymus glands, and a chemical study of the thyroids and adrenals in which the amount of iodine and epinephrine was determined from nine and one-half days until hatching. Sun made no attempts to determine the cytological significance or to trace the origin of the glands. The initiation and early development of the thyrotropic function in the developing chick embryo has been established by Martindale². Martindale concluded that the thyrotropic hormone is produced at least as early as the eleventh day of incubation, while Bradway³ assumed that the glandular functioning of the thyroid commenced after twelve days of incubation. Chen, Oldham and Geiling⁴ detected a melanophore-dispersing hormone in the hypothysis of the chick at five days of incubation. Fugo⁵ has developed a technique, and studied the effects of hypophysectomy in the chick embryo. This technique opened the way for a direct attack on the problem of endocrine relationships during early development.

Investigation Undertaken

The results presented in this investigation are concerned with the time when the embryonic endocrine glands become functional, and their possible functional interrelationship as determined from a morphological study. All embryos used were of a pure single-comb White Leghorn stock maintained by the Iowa State College Department of Poultry Husbandry. Throughout the experiment the fertile eggs were obtained from the same group of hens, which had been maintained on the following laying mash:

Ground yellow corn ......................100 lbs.
Pulverized whole oats ....................135 lbs.
Ground wheat or standard wheat middlings ...................................... 50 lbs.
Wheat bran ........................................ 50 lbs.
Alfalfa meal (bright green)............ 35 lbs.
Meat and bone meal (50%) ........... 25 lbs.
Dried milk ...................................... 15 lbs.
Soybean oil meal ......................... 40 lbs.
Corn gluten meal ........................... 10 lbs.
Ground oyster shell or high grade limestone ...................................... 15 lbs.
Bone meal ...................................... 5 lbs.
Fish meal ...................................... 15 lbs.
Salt .................................................... 5 lbs.
Cod-liver oil .................................1¼ pts.
Grain Formula
Corn (whole, shelled) ..............275 lbs.
Oats, wheat or barley ..............225 lbs.

The grain and mash were self fed. All eggs were incubated in an electrically operated Buffalo incubator.

Embryos Used

A series of 420 embryos, ranging from 12 hours of incubation to hatching, were used for the morphological studies. The technique used in preparing the glands for this purpose varied for the individual gland.

During embryogeny the primordia of the glands of internal secretion appear at different times. The following table verifies this fact.

<table>
<thead>
<tr>
<th>Gland</th>
<th>Appearance of Primordia in Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypophysis</td>
<td>43 to 48</td>
</tr>
<tr>
<td>Thyroids</td>
<td>30 to 36</td>
</tr>
<tr>
<td>Thymus</td>
<td>110 to 120</td>
</tr>
<tr>
<td>Parathyroids</td>
<td>148 to 165</td>
</tr>
<tr>
<td>Adrenals</td>
<td>90 to 96</td>
</tr>
<tr>
<td>Gonads</td>
<td>40 to 45</td>
</tr>
</tbody>
</table>

Perhaps the early appearance of the hypophyseal, thyroidal and gonadal primordia may be attributed to their regulation of body growth and to their functional interrelationship.

The first hormone known to be produced by the developing glands is the melanophore-dispersing factor. Chen, Oldham and Geiling have demonstrated this hormone in the hypophysis of the five day chick embryo. This observation is difficult to reconcile with an embryological and histological study of the hypophysis at this early period. In the first place the hypophysis is not completely differentiated, and secondly the cells do not possess secretory granules in their cytoplasm. However, the distal end of the pars glandularis is somewhat enlarged at five days, and is slightly constricted from the buccal cavity. On the basis of this observation, it may be assumed that the anterior lobe of the pars glandularis is most likely to be responsible for the production of the melanophore-expanding principle. Snyder has demonstrated the melanophore-expanding principle in the hypophysis of the Sus scrofa foetus before occurrence of the other hypophyseal hormones. In this connection Maurer and Lewis found that the melanophore-expanding principle could be present in the pars intermedia without the cells showing secretory activity. Therefore, it might be assumed that the melanophore-expanding hormone is stored during early embryonic life and liberated later, perhaps in the chick, after 462 hours or after the period at which the anterior lobe is completely differentiated.

Between 110 and 120 hours of incubation two marked morphological changes occur within the endocrine system. At this time the thyroid tissue becomes bilobed, and sympathetic cells from the sympathetic nervous system begin to migrate into the cortical primordium of the adrenal glands. It seems doubtful that these two activities are dependent on one another.

After 134 hours of development the sexual cords make their appearance in the primitive gonadal tissue. Sexual cord formation seems to take place independently of other glandular activity. The work of Fugo further substantiates this observation. Fugo found that sex differentiation is independent of hypophyseal function.

Parathyroid Glands

Between 134 and 148 hours the anterior lobes of the parathyroid glands become separated from the thyroid lobes. The anatomical change seems to be independent of other glandular activity.

After 165 hours of incubation the posterior lobes of the parathyroid glands become detached from the anterior lobes. Sinusoids appear in the adrenal glands and development of sexual cords of first proliferation ceases in the gonads. It is difficult to postulate an interrelationship between these glandular activities. However, the marked vascular growth in the adrenal glands is indicative of early function.

Between 165 and 177 hours, sex can definitely be determined microscopically.

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During the same period the large lymphocytes begin to invade the thymus primordium.

**Basophilic Cells**

The cells of the pars glandularis of the hypophysis exhibit a general basophilic staining reaction at 191 hours. These cells, according to Rann, differ from the future basophils and are considered as transitional forms. In the left ovary at this time, there is a marked increase in the number of primordial germ-cells in the germinal epithelium. It is generally recognized that the basophilic cells of the pars glandularis produce the gonadotropic hormone. On the basis of this knowledge, it seems plausible to suppose that if these embryonic basophils are producing hormone, the germinal epithelium of the left ovary would be stimulated to activity. It is difficult to justify this hypothesis when the histological picture of the testes is studied, since no activity is noted in the germ-cell-producing tissues. The left ovary may be more sensitive to the gonadotropic hormone than the testis. Fugo found that the germ-cell activity in the ovary and testis are independent of hypophyseal activity. Riddle and Schooley have found that in pigeons of less than 2.3 months, and in ring doves of less than 3.0 months, the production of follicle-stimulating hormone has not yet been initiated.

At 206 hours of development the basophilic character of the hypophysis is very pronounced. Small accumulations of colloid occur in the cytoplasm of many cells. This observation, coupled with the increased vascular development to the gland, might be considered indicative of function. At this period the adrenal glands have also become very vascular, and their chromaffin cells are situated next to the sinusoids. Therefore, if hormone is being produced by the hypophysis and adrenals, it could be made easily available for circulation.

The basophilic character of the hypophysis is maintained at 213 hours. A similar deep basophilic staining characterizes the cells of the thyroid and parathyroid glands. This deep staining is perhaps an indication that the cells will soon become secretory in nature. Further evidence that the hypophysis is now actively producing hormone occurs in the testes and ovaries. This evidence is based on the fact that the medullary cords of the ovary are rapidly increasing in length and exhibiting lumina, while the intertubular tissue of the testes is markedly increased. From the results obtained by Domm and Dennis and Venzke, it is apparent that the gonadotropic hormone stimulates the medullary portion of the embryonic ovary and the intertubular tissue of the embryonic testis. It seems that if there is gonadotropic hormone acting on the embryonic gonads, it produces an effect similar to that of the luteinizing hormone in mammals after birth.

Smith and Dortzbach and Nelson detected gonadotropic and growth hormone early during the foetal life of the Sus scrofa. The gonadotropic hormones were found to precede the growth hormone. Their studies indicated that the production of growth hormone coincides with the appearance of the basophil cells and that the gonadotropic hormone production coincides with the appearance of acidophil cells in the hypophysis.

Although the chromaffin tissue in the embryonic adrenal glands at 228 hours has no regular arrangement, the cells are slightly granular and always in contact with a venous vessel. Thus it may be assumed that the chromaffin cells are active in the production of hormone. Sun detected epinephrine in the adrenal glands at nine and one-half days of incubation. However, from a morphological point of view, the chromaffin tissue appears to become functional sometime between 206 and 228 hours of incubation.

**Acidophilic Staining**

For the first time at 240 hours, acidophilic staining cells appear in the anterior lobe of the pars glandularis of the hypophysis. This observation has been previously made by Rahn. With the appearance of acidophils in the anterior (Continued on page 94)
lobe, colloid droplets arise in some of the cells of the thyroid glands. The simultaneous appearance of acidophils in the hypophysis and colloid in the thyroid gland cells would suggest that the thyrotropic hormone is secreted by the acidophil cells. Smith and Smith\(^{15}\) found that the central part of the beef anterior pituitary, composed chiefly of basophil and chromophobe cells, is more effective in causing thyroid stimulation than the outer portion made up of acidophil and chromophobe cells. Allen\(^{16}\) found that during metamorphosis of the tadpole the basophil cells are more numerous and stain more deeply than before metamorphosis. This work supports the view that the basophil cells secrete the thyroid-stimulating principle. D'Angelo\(^{17}\), studying several species of *Anura*, observed that cytological activity of the basophil is related to hyperactivity of the thyroid, and may be concerned with the elaboration or release of a thyrotropic factor. However, Spaul and Howes\(^{18}\) concluded that the thyrotropic (metamorphic) hormone is elaborated by the acidophils. In this connection it is interesting to note that a hyperactivity of the thyroid is associated with an increased number of acidophil cells in the hypophysis\(^{19}\). The work of Rumph and Smith\(^{20}\) supports the view that thyroid secretion is not necessarily initiated by pituitary stimulation.

Recent work of Martindale\(^{2}\) points to the fact that the thyrotropic hormone is produced at least as early as the eleventh day of incubation.

Sun\(^{1}\) found that the active principle of the thyroid is detectable at the tenth day. After this time there is an increase in both relative and absolute amounts of active thyroid principle.

**Gonadotropic Substance**

Fugo\(^{3}\) observed that the cortex of both the hypophysectomized and control embryonic ovaries are similar at the tenth day. After this time Fugo observed that the ovarian cortex of the hypophysectomized embryos failed to develop properly as compared with the controls. If this work is correlated with the morphological studies, and if hormone is being produced, it might be concluded that the acidophil cells are responsible for the production of gonadotropic hormone. Fugo found that hypophysectomy had no effect on the development of the left ovarian medulla and right ovary. This observation is difficult to justify in view of the work of Domm and Dennis\(^{11}\) and Venzke\(^{12}\); since these authors were able to stimulate the embryonic ovarian medulla of the left ovary with gonadotropic substances. The question as to which cells in the hypophysis are producing the hormone might be solved by assuming that in the chick embryo the basophils produce luteinizing hormone and that the acidophils produce follicle stimulating hormone. The fact that pregnant mare's serum produces a luteinizing effect on the embryonic gonads lends support to this hypothesis. Rahn\(^{9}\) found indirect evidence that the hypophysis of the chicken exerts no influence on the gonads prior to puberty. This conclusion is not justified according to the work of Fugo\(^{5}\).

**252 Hours**

At 252 hours the most marked activity takes place in the testes where many primordial germ-cells are actively dividing. Whether this activity is the result of hypophyseal function is open to question. Fugo\(^{5}\) stated that the germ-cells are not affected, in the male, by the absence of the pituitary. Cytologically, the germ-cells in the hypophysectomized embryo do not differ from those of the controls.

Chromaffin cells of the adrenal glands at 252 hours are very granular, suggesting active secretion. Sun\(^{1}\) found that the epinephrine content of the adrenal glands invariably increased in amount with the age of the embryo. Thus, it seems that the more granular the cell cytoplasm, the greater the hormonal content.

The number of thyroid gland cells containing chromophobe colloid globules or droplets is larger at 252 hours than at 240 hours, indicating that the gland is pro-
THERE ARE

THREE SUCCESSFUL METHODS

of controlling losses due to infection incident to the shipment of cattle.

1. The injection of Hemorrhagic Septicemia Bacterin (or Mixed Bacterin Bovine, Formula 1, or Pasteurella-Pseudodiphthericum Bacterin) at least ten days before cattle are due to be shipped. This method is not recommended for use immediately before, during or immediately after shipment.

2. Immediately before shipment of cattle inject Anti-Hemorrhagic Septicemia Serum (Lockhart). Dosage: 8 to 10 c.c. per hundred pounds weight.

3. During shipment or immediately after arrival at destination inject Anti-Hemorrhagic Septicemia Serum (Lockhart). Dosage: 12 to 15 c.c. per hundred pounds.

Anti-Hemorrhagic Septicemia Serum (Lockhart) is prepared from mature bovines, known to be immune to the various “Shipping Fevers” of cattle.

The above recommendations are in keeping with the discussion of this subject appearing in the 1942 Yearbook of the U. S. Department of Agriculture, “Keeping Livestock Healthy,” pages 526-532.

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ducing increased amounts of thyroid principle. This morphological observation is verified by the chemical studies made by Sun. Acidophil cells continue to increase in number within the pars glandularis of the hypophysis. This cellular increase is associated with continued thyroid growth and the appearance of thyroid follicles at 260 hours.

At 272 hours the acidophil cells appear in the posterior lobe of the pars glandularis. This cellular increase is associated with changes in the parathyroid glands, in which the cells are becoming acidophilic in nature. Perhaps this change marks the production of an active principle by the parathyroids. It also seems plausible to assume that the cortical cells of the adrenal are now secretory in function, since the cytoplasm is rather granular. Thus, it might be possible to assume that the acidophil cells are responsible for initiating the function of the parathyroid and adrenal cortex. However, Fugo observed that the parathyroid and adrenal glands are not affected by hypophysectomy during embryonic development.

**Ossification**

Ossification can be observed in many areas of the skeleton at 284 hours. If the parathyroid glands are producing an active principle at this time for the stimulation of ossification, it must be assumed that the acidophilic staining cells are responsible for the active principle.

At 295 hours of development the acidophil cells of the hypophysis show an increase in number and amount of cytoplasmic granulation. As a result of this cellular activity, the thyroid, parathyroid and adrenal cortical cells show marked stimulation.

At 307 hours of development the interstitial cells first appear in the testes. Fugo has shown that the lack of hypophyseal stimulation is the cause of the failure of intertubular material to develop. Thus, it may be assumed that the hypophysis is functioning during embryonic life.

The acidophil cells of the pars glandularis become completely differentiated in both lobes at 319 hours. Coincident with this observation is the appearance of a second kind of cell in the parathyroid glands. This cell stains lightly with eosin and possesses a large, poorly chromatic nucleus. At the same time the thymus presents a well developed cortical and medullary zone. The thymus may now be assumed to be functional. According to Fugo, the thymus develops independently of the hypophysis. Perhaps the above observation is merely coincidence. It is interesting to note that the differentiation of the parathyroid glands keeps pace with the differentiation of the acidophilic elements of the pars glandularis.

Basophil and acidophil cells appear in the pars tuberalis at 331 hours. The rather complete differentiation of the hypophysis at such an early period is responsible for producing growth of the entire endocrine system. The role which the active principle of the glands is playing with respect to each other, and to body growth in general, cannot be ascertained from a morphological study. However, from this time until hatching, the rate of body growth is greatly augmented, indicating a combined function of all the endocrine glands. It does not appear, from the work of Fugo, that the thyroid is directly concerned with body growth. By injecting thyrotropic substance into hypophysectomized embryos Fugo was able to restore the thyroid to normal appearance. This had no noticeable affect on body growth. It is apparent that the growth promoting factor of the pars glandularis is mainly responsible for body growth.

**Cytological Changes**

Marked cytological changes occur in the pars glandularis from 388 to 450 hours of development. From the work of Severinghaus it may be concluded that during this period the acidophils reach their peak of granular accumulation. If cytoplasmic granulation is an index of secretory activity, it may be assumed that the pars glandularis is most active during this period. From 462 hours through hatching, the two lobes of the pars glandularis become cytologically distinct; the anterior
lobe possesses acidophil, basophil, and chromophobe cells, while the posterior lobe contains basophil, chromophobe, and weakly staining acidophil cells. Kleinholz and Rahn determined the intermedin content of the chicken pars glandularis and found that the anterior lobe contained the greatest quantity of this factor. Therefore, since the light staining acidophil cells predominate in the anterior lobe it seems plausible to assume that they are responsible for the production of the intermedin factor. Since there is no pars intermedia in the hypophysis of the chicken, it must be assumed that the anterior lobe of the pars glandularis substitutes, at least in part, for its function.

**Parathyroid Glands**

At 400 hours the parathyroid glands present three types of cells differentiated on the basis of staining and structure. One may be described as a clear cell since the nucleus and cytoplasm stain poorly. A second stains lightly acidophilic, is finely granular, and possesses a nucleus which is smaller and less vesicular than that of the clear cell. A third cell type occurs singly or in groups and contains large amounts of granular cytoplasm which stains intensely with acid dyes. The nucleus, however, is much smaller than in the other cell types, and stains deeply basophilic. It is difficult to ascertain that the three types of cells described are producing different active principles. It would seem more plausible to assume that they are cells in different stages of secretory activity. This statement is based on the fact that these cell types increase and decrease rather markedly in numbers throughout the remainder of the incubation period.

Little histological change takes place in the other endocrine glands from 400 hours until hatching. Growth, both of the body and of the glands, is the only striking change which occurs during this time interval.

Rahn stated that in the chicken the gonads do not elaborate sex hormone until puberty as evidenced by the lack of comb growth. This observation is further substantiated by Domn and Dennis and Venzke. These workers stimulated the intertubular tissue of the embryonic testes and the medulla of the left embryonic ovary, but the head furnishings showed no change over those of the controls. Therefore, it must be assumed that the embryonic gonads do not produce sex hormone.

**Summary and Conclusions**

The morphogenesis of the hypophysis, thymus, thyroid, parathyroid and adrenal glands, ovaries and testes has been studied by means of serial, sagittal, transverse and frontal sections, taken at approximately twelve hour intervals from the twelfth hour of incubation until hatching. A series of 420 chick embryos was used. The results presented are concerned with the time when the embryonic glands become functional, and their possible functional interrelationship.

Indirect evidence is presented to substantiate the hypothesis that gonadotropic hormone is secreted by both the basophilic and acidophilic cells in the hypophysis beginning at 213 hours of incubation.

The rate of growth is most rapid after appearance of the acidophilic cells in the hypophysis.

Indirect evidence is presented to substantiate the hypothesis that thyrotropic hormone is produced by the acidophil cells in the hypophysis at 240 hours of incubation.

The appearance of acidophil cells in the posterior lobe of the pars glandularis at 272 hours is associated with possible functioning of the parathyroid and adrenal cortical cells.

Indirect evidence is reported to substantiate the hypothesis that the light staining acidophil cells in the anterior lobe of the pars glandularis of the hypophysis produce the intermedin factor.

The thymus gland appears to become functional at 357 hours of incubation. No evidence was obtained to indicate that thymic development is dependent on the function of other endocrine glands.

At 165 hours the morphological sexual
differences become apparent in the gonads. No evidence was obtained to indicate that sex hormone is produced during the incubation period.

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ENCEPHALOMYELITIS

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be found congregated in or about cells as in the yolk sac cells. The bodies appear like cocci to some extent, resembling the pleuropneumonia organisms. They do not resemble their protozoa, and their shape, size, and especially location, indicate that they are not Rickettsia. (The authors are indebted to Dr. Alfred M. Lucas, Zoology Department, Iowa State College, and Dr. Cornelius B. Philip, U. S. Public Health Service, Hamilton, Montana, for examination of slides.)

BIBLIOGRAPHY


EDITORIALLY

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We must be our own teachers. We must be willing to do our own thinking (our veterinary education should cultivate that tendency in us). We should look about us with intelligent curiosity. We should examine the issues of the day from all angles. We should try to gain more information and understanding all the time, and avoid sinking into narrow prejudices. If we are to prepare for the veterinarian of tomorrow, we must be able to meet Tyndall’s challenge, “Is he a man of information and good sense?” When we can do that, we will find, with him, that “knowledge once gained casts a light beyond its own immediate boundaries.”

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