Phosphomonoesterase Content and Localization in the Meso- and Metanephros of the Chick Embryo

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With two plates (figs. 6 and 7)

SUMMARY

Chemical determinations were made of the alkaline and acid phosphatase content of meso- and metanephros of chick embryos between 4 days of incubation and 6 days after hatching. Histochemical localization of alkaline phosphatase and of ribonuclease-removable basiphilia was also studied in these organs.

A study of the data obtained suggest the following conclusions:

1. The acid and alkaline phosphatase activity of the mesonephros increases from the 4th to the 16th day, decreasing from then onwards.

2. The acid and alkaline phosphatase activity of the metanephros increases from the 11th to the 16th day, decreasing slowly to the 20th day. A rapid rise is observed after hatching.

3. The alkaline phosphatase activity that is diffusely located in the cells of recently formed secretory tubules of the meso- and metanephros polarizes to the brush border zone later on.

4. Ribonuclease-removable basiphilia, abundant in the mesonephric tubules up to the 6th day of incubation, decreases from then onwards.

5. The metanephric blastema and tubules present a high concentration of ribonuclease-removable basiphilia up to the 13th day. From then on its content decreases in the whole organ with the exception of certain groups of cells still in differentiation.

The possible correlation between these findings and meso- and metanephros differentiation and function is discussed.

INTRODUCTION

ALTHOUGH several papers have been published on the chick embryo mesonephros, the time of beginning and end of its function still remains in doubt. Considerably fewer data can be found in the literature concerning the metanephros.

In an attempt to investigate this problem a study of the acid and alkaline phosphatase content and alkaline phosphatase localization was undertaken in the meso- and metanephros of chick embryos. Previous results in this field were reported by Moog (1944), who studied the acid and alkaline phosphatase distribution in chick embryos up to the 8th day of incubation. Consequently her data are restricted to the early development of the mesonephros.

ABSOLUTE WEIGHT OF MESO AND METANEPHROS

MESONEPHROS ———— METANEPHROS

DAYS 0 2 4 6 8 10 12 14 16 18 20

Fig. 1. Variation in the absolute weight of chick meso- and metanephros.

RELATIVE WEIGHT OF MESO AND METANEPHROS

ORGAN WEIGHT IN MG PER BODY WEIGHT IN GR.

MESONEPHROS ———— METANEPHROS

DAYS 0 2 4 6 8 10 12 14 16 18 20

Fig. 2. Relative weight of chick meso- and metanephros. Observe substitution of meso- by metanephros around the 13th day.
Brachet’s toluidin blue ribonuclease technique was applied to sections to study the distribution of the ribonuclease-removable basiphilic substance in embryonic chick kidneys in an attempt to correlate this substance with the differentiation of these organs.

**Material and Methods**

Eggs of New Hampshire Red chickens were incubated for desired periods at 39° C. The weight curves were made from meso- and metanephros of chicks of several ages fixed in Zenker-formalin, washed in water for 24 hours, and stored in 70 per cent. alcohol. No fresh weight determinations could be made owing to the difficulty of dissecting the kidneys in young stages. The histochemical localization of alkaline phosphatase was made using Gomori’s (1939) method on iced 80 per cent. alcohol-fixed material. The sites of alkaline phosphatase activity appear stained by this technique in black or dark brown. The shrunken appearance of the tissues is due to the fixation. Chick embryos ranging from 3 to 20 days’ incubation were used, and in some instances chicks were used 6 days after hatching. A minimum of four embryos was used for each age studied.

Brachet’s ribonuclease method was used as described by Stowell and Zorzoli (1947).

Quantitative determinations of acid and alkaline phosphatase were made by the method described by Binkley, Shank, and Hoagland (1944). Since often only small amounts of tissue were available, this method was adapted for our purposes by using half of the amounts of substrate and reagents described in the original paper. This procedure permitted the use of 5 mg. of tissue (wet weight) for each determination. Readings were made in a Klett colorimeter.

The results presented are the averages of at least four determinations of each age. Incubation time was 30 minutes for the alkaline phosphatase and 60 minutes for the acid phosphatase. In addition, slides of Zenker-formalin material were stained with haematoxylin and eosin and compared with those used for histochemical studies.

**Results**

Results obtained by weighing the meso- and metanephros are summarized in figs. 1, 2, and 3. Fig. 1 shows that the absolute weight of the mesonephros increases gradually to the 14th day and from then on declines slowly. The relative weight curves (fig. 2) show that on approximately the 10th day the mesonephros stops growing proportionally to the body weight, while the metanephros grows very rapidly until the 14th day, its growth becoming from then on roughly proportional to the embryo’s body weight.

Fig. 3 indicates that the relative total kidney weight grows gradually to a maximum at 12 days and decreases from then on.

**Biochemical results:** Results obtained in chemical determinations of phosphatases are summarized in Table 1 and in figs. 4 and 5. These figures suggest
Fig. 3. Relative total kidney weight. Observe relatively small variations during the period observed as compared to changes in absolute weight.

Fig. 4. Chick meso- and metanephros alkaline phosphatase activity.
Fig. 5. Chick meso- and metanephros acid phosphatase activity.

TABLE 1. Phosphatase Activity of Meso- and Metanephros*

<table>
<thead>
<tr>
<th>Days</th>
<th>Mesonephros</th>
<th>Metanephros</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acid phosphatase</td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td></td>
<td>x = 0.315 s = 0.041 cv = 13.0%</td>
<td>x = 0.24 s = 0.060 cv = 20.7%</td>
</tr>
<tr>
<td>8</td>
<td>0.28 x = 0.460 s = 0.023 cv = 5.0%</td>
<td>0.44 x = 0.495 s = 0.050 cv = 12.1%</td>
</tr>
<tr>
<td></td>
<td>0.34 x = 0.56 cv = 8.2% 0.48</td>
<td>0.80 x = 0.870 s = 0.150 cv = 16.1%</td>
</tr>
<tr>
<td>16</td>
<td>0.52 x = 0.400 s = 0.010 cv = 25.0%</td>
<td>0.62 x = 0.620 s = 0.163 cv = 26.3% 0.66</td>
</tr>
<tr>
<td>20</td>
<td>0.30 x = 0.400 s = 0.010 cv = 25.0%</td>
<td>0.62 x = 0.620 s = 0.163 cv = 26.3% 0.66</td>
</tr>
<tr>
<td>26</td>
<td>0.56 x = 0.570 s = 0.034 cv = 5.9% 0.56</td>
<td>1.56 x = 1.630 s = 0.114 cv = 6.9% 0.56</td>
</tr>
</tbody>
</table>

* Expressed in mg. of phenol liberated by 100 mg. of organ (fresh weight).
Legend: x = mean. s = standard deviation. cv = variation coefficient.
a gradual increase of the acid and alkaline phosphatase content of the mesonephros to a peak about the 16th day and a more rapid decrease from then to the 20th day.

It is interesting to observe the increase of the acid and alkaline phosphatase content of the metanephros from the 11th to the 16th day and subsequent decrease to the 20th day followed by an increase after the hatching and consequent feeding of animals. Another point of interest is the fact shown in the figures that the changes in both phosphatases are rather alike for the same organ and that apparently the alkaline phosphatase content always presents more intense changes than the acid phosphatase.

**Histochemical results:** The morphology of the meso- and metanephros varies greatly according to the zone sectioned. This is due to the cranio-caudal gradient of differentiation which these organs present. Descriptions made here are from approximately the middle of the gland. In the 3-day embryos the mesonephros is still differentiating, and adjacent to the Wolffian duct we see an aggregation of differently shaped cells that stain darkly by Gomori's alkaline phosphatase method (fig. 6, A). This region will later give rise to the mesonephric tubules, as shown in fig. 6, B, where in a section of the caudal portion of the mesonephros of a 4-day embryo the tubules are being formed by the mesonephric blastema. It was observed that the newly formed secretory tubules have a strong alkaline phosphatase activity in the cytoplasm. In a section of a more cranial region (fig. 6, C), one can see the formed tubules and a

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**Fig. 6 (plate).** The photomicrographs represent sections treated by Gomori's alkaline phosphatase technique. The scales represent 50 μ.

A. Section of the mesonephric region of a 3-day chick embryo. Observe Wolffian duct with a strong reaction in the cell nuclei and the mesonephric blastema below it with dark-staining cells indicating high alkaline phosphatase activity. Six hours' incubation. × 160.

B. Section from a 4-day embryo. Observe strong diffuse alkaline phosphatase activity in newly formed tubules and blastema of the caudal region of mesonephros. Six hours' incubation. × 160.

C. Section from the same embryo in a more cranial region. There is a localization of alkaline phosphatase activity in the secretory tubules with a beginning of its polarization to the brush border region. Four hours' incubation. × 240.

D. Mesonephros and gonad of a 6-day embryo. Observe strong positive alkaline phosphatase reaction in the secretory tubules with no reaction in the cytoplasm of the cells of the excretory tubules and glomeruli. Five hours' incubation. × 40.

E. Mesonephros from an 8-day embryo. Observe greater amount of tubules and glomeruli when compared with the 6-day embryo. A strongly positive reaction may be observed in the Müllerian duct. Five hours' incubation. × 40.

F. Section of an embryo of the same age showing the metanephric blastema with a strongly positive reaction and the beginning of tubule formation. Two hours' incubation. × 40.

G. Meso- and metanephros of a 10-day embryo. Observe increase in the tubules formed by the metanephric blastema. The mesonephric tubules present an increase in alkaline phosphatase activity when compared with the 6- and 8-day embryos. Five hours' incubation. × 40.

H. Mesonephros of an embryo of the same age. An intense reaction with polarization towards the apical region is visible in the secretory tubules. Glomeruli and excretory tubules present practically no activity in the cytoplasm of their cells. Five hours' incubation. × 160.

I. Metanephros of an 11-day embryo. Observe abundance of tubules with an alkaline phosphatase reaction. The blastema tissue is still present and shows an intense reaction. Five hours' incubation. × 120.
difference in alkaline phosphatase activity between secretory and excretory tubules. Apparently, at this early age the secretory tubules already have a higher alkaline phosphatase activity than the excretory ducts. A careful examination of this figure will also show a beginning of polarization of the alkaline phosphatase activity to the brush border region. Glomeruli do not show alkaline phosphatase activity in sections of any of the embryos studied.

In the 6-day embryos there is a well-formed mesonephros in which the secretory and excretory tubules are easily distinguished by the intense alkaline phosphatase activity in the former and lack of activity in the latter. A polarization of alkaline phosphatase activity to the brush border region of the cell is quite evident in fig. 6, D.

The 8-day embryo presents a more highly developed mesonephros with more tubules and glomeruli than those previously described (fig. 6, E). Here one can see that the alkaline phosphatase activity has the same distribution as previously described. In fig. 6, F (also of 8 days) it is interesting to observe the metanephric blastema surrounding the metanephric tubules that are being formed. This blastema is composed of closely packed and variously shaped cells that stain darkly with Gomori's technique. This aspect is very striking if one compares these cells with adjacent mesenchyme cells that present practically no alkaline phosphatase activity. In sections of 10-day embryos (fig. 6, G and H) a picture very similar to that already described may be observed, although the secretory mesonephric tubules present a more intense alkaline phosphatase activity than in the 8-day embryo. The metanephros is more advanced in development and a greater quantity of newly formed tubules and blastema may be seen. These tubules that are being formed present a high concentration of alkaline phosphatase in their cytoplasm. The

FIG. 7 (plate). A–D represent sections treated by Gomori's alkaline phosphatase technique; E–G stained with toluidine blue. The scales represent 50 μ.

A. Section of a 15-day embryo. Mesonephros in the upper part presenting a very strong reaction in the secretory tubules. The metanephros presents a distinct polarization of a strong alkaline phosphatase activity in the apical region of the secretory tubule cells. Five hours' incubation. ×200.

B. Mesonephros of a 20-day embryo. Observe disintegration of the mesonephric ducts with shedding of particles presenting alkaline phosphatase activity. Five hours' incubation. ×200.

C. Section from a metanephros of a 20-day embryo. Observe a distinctly diminished alkaline phosphatase activity with a narrow band of activity in the brush border zone. This is particularly evident if we compare it with A or D. ×200.

D. Metanephros of a 6-day-old chick. Observe intense and polarized activity in the secretory tubule cells. Glomeruli and excretory ducts present practically no activity in the cytoplasm of its cells. Five hours' incubation. ×200.

E. Mesonephros of a 5-day embryo presenting its tubules darkly stained by toluidine blue. ×200.

F. Section of an 8-day embryo mesonephros. Observe lighter staining of the tubules. The Müllarian duct cells present their basal region rich in R.R.B. ×40.

G. Meso- and metanephros of an 11-day-old chick embryo. Compare the dark staining of the metanephric blastema and tubules (in the lower part) with the lightly stained mesonephros (in the upper part). ×120.
metanephros of the 11-day embryo is still more advanced in development and has several thin-walled tubules with only moderate amounts of alkaline phosphatase. In this stage polarization of alkaline phosphatase activity in the brush border region is just beginning (fig. 6, i). The mesonephros shows a strong and polarized alkaline phosphatase reaction in its secretory tubules.

Sections of 15-day embryos are characterized by the very intense alkaline phosphatase activity of the secretory tubules of the mesonephros and by the increase of this activity and its polarization in the secretory tubules of the metanephros (fig. 7, a). Alkaline phosphatase distribution in meso- and metanephros in the 17-day embryo presents no visible difference from the one just described.

A distinct change is apparent, however, in the mesonephros of 20-day embryos, where a clumping of the secretory tubule cells can be seen, as well as the shedding of alkaline phosphatase active particles (possibly cellular parts) into the tubular lumen (fig. 7, b). In the metanephros of embryos of this age a decrease in the activity of alkaline phosphatase can be observed in the brush border region of the secretory tubules (fig. 7, c). This diminution is very clear if one compares these slides with those of a 15-day embryo or, better, with those of a chick 6 days after hatching (fig. 7, d). Here the apical pole is very intensely stained along a relatively large band, indicating a greater alkaline phosphatase activity.

The study of slides stained with toluidin blue, with and without previous treatment with ribonuclease, showed in 5-day embryos a concentration of ribonuclease-removable basiphilic substance (R.R.B.) in the mesonephros. The tubules stained intensely although not to the same extent (fig. 7, e). There is no localized and constant intracellular distribution of R.R.B. The glomeruli always stained less than the tubules. Other organs which stained deeply at this age were the spinal cord, spinal ganglia, and liver.

In an 8-day embryo the mesonephric tubules stain with a much lighter colour, indicating a marked diminution of R.R.B. at this age. The Müllerian duct, however, presents a band of R.R.B. in the basal part of its cells (fig. 7, f). From this age onwards the R.R.B. of the mesonephros declines and becomes very scarce.

Sections of an 11-day embryo show the metanephric blastema and newly formed tubules staining darkly when compared with the adjacent mesonephros (fig. 7, g). In 15-day embryos the whole metanephros stains more darkly than the mesonephros, but several localized points situated in the periphery of the metanephros may be seen that stain still more intensely. These regions correspond to points where differentiation is still going on, as one can ascertain in haematoxylin-eosin stained slides.

**Discussion**

Wilmer (1943) demonstrated in normal and hydronephrotic kidneys a relation between phosphatase activity and kidney function. Reports in the literature as to the function of phosphatases in the kidney have been con-
flicting and were reviewed by Moog (1946), who stated on the basis of the evidence available: 'The conclusion that phosphatase is integrally concerned in renal function is thus inescapable, and the hypothesis that the enzyme is part of a glucose reabsorbing cycle is in good agreement with the facts.' This contention has been recently confirmed by March and Drabkin (1947), who demonstrated a correlation between blood sugar concentration and alkaline and acid phosphatase activity in the kidney. Furthermore, they obtained inhibition of both these enzymes by phloridzin in vivo and in vitro.

The possibility that the phosphatase activity of kidneys is correlated with the organ's function seems, therefore, very probable. The following discussion is based on this assumption.

Our data suggest that the beginning of mesonephric activity is between the 4th and 5th day of incubation, for at that period there is a concentration of alkaline phosphatase activity in the brush border zone of the secretory tubules. These findings concerning the beginning of secretion agree with Bakounine (1895), Lillie (1908), Atwell and Hanan (1926), Hanan (1927), Boyden (1927), Hurd (1928), Patten (1929), Chambers and Kempton (1933), Schneider (1939), and Moog's (1944) views on this subject. For a summary of their results see Table 2. It seems probable from our data that the mesonephros attains the peak of its activity between the 14th and 16th days. From then on the mesonephros not only loses weight, but its alkaline and acid-phosphatase activity are diminished. At hatching-time, however, there is still an appreciable amount of alkaline and acid phosphatase activity left. Our histochemical results for alkaline phosphatase agree with those of Moog (1944). These results do not confirm those of Lillie (1908), Hurd (1928), or Patten (1929), who place the beginning of the diminution of mesonephric activity between the 9th and 12th days of incubation. They are, however, in agreement with Fredericia (1912), Atwell and Hanan (1926), and Chambers and Kempton (1933), who state that the mesonephros activity declines only after the 16th to 18th days of incubation. Gersh (1937) observed that the mesonephros eliminates phenol red and ferricyanide up to at least the 14th day. He stated further that the decline of mesonephric activity, far from being abrupt, is a slow process. Our observations seem to corroborate this view.

As to the time of the onset of metanephros activity, our data are in accordance with those of a number of authors who contend that secretion begins after the 11th day of incubation (for a summary, see Table 2). At this date secretory tubules with alkaline phosphatase activity are present and our biochemical observations show a relatively rapid rise of alkaline and acid phosphatase activity at the same stage.

It is interesting to note that the peak of activity of both phosphatases is at about the 16th day followed by a decline from then on to the hatching time (figs. 4 and 5). A study of the literature showed that during this period the capacity of the metanephros to concentrate dyes is frequently lower than that of the mesonephros. These facts coincide with the reduction of the relative total kidney weight observed after the 14th day (fig. 3) and might suggest a
The only known metabolic change in the chick embryo that could be correlated with these facts is the marked diminution of protein metabolism observed by Fredericia (1912) after the 16th to 17th day, Fisk and Boyden (1926) after the 14th day, and Needham (1926) after the 11th day.

The rapid increase of acid and alkaline phosphatase activity after hatching and consequent feeding of the animal suggests that there might be a correlation between food ingestion and phosphatase content in the metanephros. It seems to us that it would be of interest to feed or inject several foodstuffs of different nature in recently hatched chicks and study their action on the alkaline and acid phosphatase activity of the metanephros.
It is conceivable that the phosphatases play a role not only in the functional activity of the kidneys but also in their differentiation.

The presence of a strong alkaline phosphatase activity, diffusely located in the cells of the meso- and metanephric blastema and newly formed tubules, suggests such a correlation. Other examples of this fact were presented by Moog (1944).

The results obtained with Brachet’s ribonuclease technique show a greater amount of ribonuclease-removable basophilia in the early mesonephros (before the 6th day of incubation) when compared with the same organ later on. This finding suggests a correlation between the mesonephros differentiation and its R.R.B. content. We could thus strengthen Caspersson and Thorell’s (1941) conclusions obtained with the ultra-violet absorption method. The metanephros presents, during its differentiation, concentration of R.R.B. in the blastema and newly formed tubules. This is particularly evident during the period between the 7th and 12th days of incubation and decreases from then on with the exception of certain groups of cells still in differentiation. These groups of cells persist up to the 20th day of incubation. Owing to this fact we are led to believe that differentiation is apparently more rapid and uniform in the mesonephros than the metanephros.

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