

THE MORPHOGENETIC FUNCTION OF A VESTIGIAL ORGAN IN THE CHICK

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(With One Plate)

INTRODUCTION

THE pronephros of the chick is usually considered to be a vestigial organ. It only appears for a short period during early development and it is never functional as an excretory organ. From it, however, the primordium of the Wolffian duct is formed, by the confluence of the segmented pronephric tubules to a continuous strand of cells. This strand lengthens and extends into the posterior part of the body, although the pronephros itself does not develop behind about the sixteenth somite. At a later stage, the mesonephros, which is the functional excretory kidney during most of embryonic life, appears in close connexion with the Wolffian duct in the region from about the fourteenth to the thirtieth somite (Lillie, 1919). The purpose of the experiments recorded in this paper was to examine the morphogenetic mechanisms involved in this sequence of events. All the experiments on chicks, except a few which are specially described, were performed on embryos cultivated *in vitro* in the usual way. A few experiments have been made on embryos of *Rana fusca* and are described later.

THE EXPERIMENTS

(a) *Time of determination of the pronephros*

The determination of the pronephric tubules probably occurs at a stage corresponding to very early neural plate formation. At least it cannot occur earlier, since it is clear from previous work (Waddington & Schmidt, 1933; Abercrombie & Waddington, 1937; Rawles, 1936) that the regional determination of the mesoderm is not complete in the primitive streak stage. In the early neural plate stage, which, of course, occurs earlier in the anterior parts of the embryo than in more posterior parts, in accordance with the well-marked anterior-posterior gradient of differentiation in the chick, the mesoderm seems to be already determined as either somitic mesoderm, intermediate cell mass, or lateral plate. The second of these seems to be capable of producing pronephric tubules in the absence of either the first or the last.

If a longitudinal cut is made slightly to one side of the neural plate in a region of the embryo which is in the early neural plate stage, the intermediate cell mass may be completely separated from the lateral plate; and pronephric tubules are formed during later development (Pl. I, fig. 1). If the cut is rather nearer the midline the intermediate cell mass may be separated from the somites and joined only to the lateral plate; again pronephric tubules may be developed (Pl. I, fig. 2). In these embryos there is, however, always a little neural tissue included with the lateral plate and intermediate cell mass, since the neural plate extends, when it is flat, slightly further laterally than the somitic mesoderm. The possibility therefore arises for both sorts of experiments that the development of pronephric tubules may be dependent on the presence of neural tissue. The anatomical relations make this rather unlikely; the distortion of cut embryos often carries the neural tissue some distance away from the tubule, which nevertheless appears in its normal position in the mesoderm. The suggestion cannot, however, be ruled out, and Gr  newald (1935) has suggested that the neural plate is also responsible for the induction of the somites from the underlying mesoderm. The evidence on this question seems to be still somewhat undecided, and further consideration of it will be postponed till experiments now in progress have yielded definite results.

(b) *The elongation of the Wolffian duct*

From purely descriptive evidence it has been concluded (cf. Lillie, 1919) that the elongation of the Wolffian duct towards the posterior is due entirely to the multiplication of its own cells, and that no other cells are added to it from regions posterior to the last pronephric tubule. This is fully confirmed by the experimental evidence. If a transverse cut is made across the axis of an embryo of about 8–12 somites, posterior to the last pronephric tubule and posterior also to the end of the developing Wolffian duct, no Wolffian duct ever appears behind the cut. This is most clearly shown in embryos which have been cut only on one side; on the unoperated side, which acts as a control, the duct appears as usual in the posterior region, while on the cut side no duct is developed posterior to the cut (Pl. I, fig. 3). The intermediate cell mass develops to some extent independently of the presence or absence of the duct, so that even when the duct is absent a small cleft is formed into which the duct would fit if it were there.

(c) *The formation of the mesonephros*

The mesonephros does not develop in clearly recognizable form till about the 29–30 somite stage. The embryos described above could hardly be kept alive *in vitro* long enough to reach this stage. The best preserved, however, seemed to show, on the cut side from which the Wolffian duct was missing, a failure of the condensation of nephrogenous tissue from which the mesonephros would later develop. Attempts were made to check this. First, the operation was repeated on older embryos. But the duct extends rather far posteriorly in quite early stages, so that the part posterior to the end of the duct represented a more posterior level of the embryonic axis than it would in a younger stage, and therefore required just as long to develop

satisfactory mesonephric tubules. The operations were therefore done on 8–12 somite embryos and an attempt was made to lengthen the period of cultivation; after a day's incubation of the entire embryo, the posterior part of the body, extending from the tail to just anterior to the cut, was transferred to a new clot and incubated for a further day. In some of these embryos the evidence was more convincing; there were several cases in which the mesonephros is lacking on the side which lacks the duct, and present on the side where the duct is present. The development of the mesoderm, however, was by no means satisfactorily typical. There was a considerable tendency for the somites to break up into mesenchyme as it does in cultivated mammalian embryos at an earlier stage of culture (Waddington, 1937), and the anatomical relations were considerably distorted by the mechanical conditions.

Satisfactory evidence of the dependence of the mesonephros on the Wolffian duct was finally obtained by operating on embryos in the shell. The embryos were slightly stained with neutral red for greater visibility, and a cut was made through an opening in the shell. The cut, as before, was on one side only, running from the midline of the embryonic axis transversely out into the area pellucida. It was found advantageous to pull the edges apart slightly and allow a little yolk to flow through, as this hinders the joining together of the cut edges. The window in the egg was then closed with cellophane and the egg incubated in a damp atmosphere. The embryos were operated on at about the 8–12 somite stage, and fixed after 2 days. The mesonephros is well developed on the control side, but in those embryos (eight in number) in which the operation has been successful in preventing the extension of the Wolffian duct, no fully developed mesonephros occurs (Pl. I, fig. 4). Even in the absence of the duct, however, there do occur a few small isolated patches of nephric tissue, which are very insignificant in size and poorly developed; no lumen has been found in them (Pl. I, fig. 5). There can be no doubt that the Wolffian duct plays the major part in inducing the mesonephros, but it probably usually co-operates with other influences of lesser importance, which in its absence can only produce the feeble patches of tissue just mentioned. This conclusion relates only to an early stage in the development of the mesonephros, and, as will be pointed out in the discussion, it is possible that more mesonephros would have been found in later stages of development of the operated embryos.

DISCUSSION

In some form or other, the fact of recapitulation is accepted by most biologists. In the ontogeny of a highly evolved animal, organs which were important and functional in its ancestors may be found in a modified and sometimes rudimentary form. The question arises why an organ, which has been reduced to a mere functionless vestige, should be retained at all and not entirely lost. Kleinenberg (1886) was the first to suggest that those vestigial organs which are thus retained (and it should be noted that not by any means all of them are) have in fact still got a function in preparing the way for later-evolved organs which play an essential role in the animal's life-processes. In his review of the subject, Needham (1931) made the

idea more precise and suggested that a vestigial organ may be retained because it provides a morphogenetic stimulus which induces the formation of a later evolved organ. Thus it might be suggested that the notochord, which is still functional in the amphibian larva as part of the skeleton, has become vestigial in the chick and the mammal and is retained only for its function of inducing the neural plate (Waddington, 1930, 1937); but the case is not quite clear, since the neural induction is actually performed by the axial mesoderm as a whole rather than by the notochord in particular. A much clearer example is provided by the facts about the relations of the pro- and mesonephros related above.

The pronephros of the chick, from the point of view of metabolic physiology, is a completely vestigial organ with no known function. But we have seen that it alone gives rise to the Wolffian duct, which plays the most important part in causing the formation of the functional mesonephros, without which embryonic life would hardly be possible. Clearly we cannot simply dismiss the pronephros as functionless. We must distinguish between "synchronic functions", all those which concern the day to day living of an animal, its feeding, movement, metabolism, etc., and "diachronic functions" which are concerned with long period changes, development, heredity, evolution, etc. The pronephros has a diachronic function but no apparent synchronic function.

There are indications that the chick is already beginning to evolve an alternative mechanism of formation of the mesonephros which, if more highly developed, would allow it to dispense with the pronephros as an inducing agent, though the necessity to produce a Wolffian duct in some way would still be operative. Thus we find that slight traces of mesonephros can be formed even in the absence of the duct. This betokens, in the chick, no more than a feeble degree of "doppelte Sicherung", or capacity for self-differentiation.

It would be very interesting in this connexion to have comparative data on the relations between pro- and mesonephros in other phyla. In most forms, the question seems not yet to have been investigated, but Miura (1931) and Shimasaki (1931) have published the results of some experiments performed on Anura. Their results, however, were not in agreement. Miura found that if a large enough piece of the pronephric duct was removed, no regeneration of the duct occurred, and the mesonephros failed to appear on the operated side. Shimasaki, on the other hand, found that a somewhat reduced mesonephros could develop even in absence of the pronephric duct. He was inclined to attribute the difference between his results and Miura's to the fact that Miura fixed his embryos at an earlier stage, and he suggests that if Miura had waited longer, some mesonephros would have developed in his larvae also. For purposes of comparison with the experiments on the chick described in this paper Miura's results seem to be more relevant than Shimasaki's, since, the chicks also, owing to the technical difficulty of keeping them alive, were killed in a fairly young stage.

Miura's experiment has been repeated on embryos of *Rana fusca*, from which the posterior end of the pronephric swelling was removed in the young tail-bud stage. The embryos were fixed in an early stage of mesonephros formation, and the

results fully confirm those described by Miura. An example is illustrated in Pl. I, fig. 6. It thus becomes clear that in the Anura also the pronephric duct plays a large part in the initial induction of mesonephros development. The morphogenetic function of the chick pronephric duct is therefore one of some phylogenetic antiquity. At the same time, both Anura and birds show some degree of "doppelte Sicherung" for the mesonephros; in birds traces of mesonephros can appear even in early stages without the help of the pronephric duct, while in Anura small mesonephroi are present in later stages but seem to be completely absent in early stages. It is not easy to compare the degree of "doppelte Sicherung" shown in these two cases, but it is at least clear that the mechanism evolved by the chick is not very much more independent of the duct than that of the frog.

If we try to consider the conditions in the phylogenetically primitive condition, we may probably assume that the pro- and mesonephros were originally continuous, and two possibilities arise: (1) that each nephric tubule sends off a shoot, which contributes to the duct, and which induces the tubule in the next segment; and (2) that all the tubules are capable of independent differentiation. If the second suggestion were true, it seems rather peculiar that the more posterior tubules, which develop into the mesonephros in higher forms, have lost the capacity for self-differentiation and have come to require an inducing stimulus; but this change, if it occurred, might perhaps have been correlated with the change which has led to the separation of the continuous row of tubules into two distinct series. The first alternative is perhaps more plausible, but as yet no definite cases are known of the dependence of metamerically segmented organs on transmission from segment to segment; the mechanism of metameric segmentation is, however, not sufficiently understood for the possibility to be excluded.

SUMMARY

1. The pronephros opposite any given somite in the chick is probably determined just before the early neural plate stage at that level, and thereafter develops independently of the somite or lateral plate mesoderm.

2. The Wolffian duct, which is first formed by the union of the pronephric tubules, grows backwards by the multiplication of its own cells. If this elongation is checked by a transverse cut made posterior to the end of the duct, the duct does not appear behind the cut.

3. In the absence of the Wolffian duct, the mesonephros is not properly developed, appearing only as very small patches of tissue with no lumen. The Wolffian duct may therefore be said to induce the mesonephros, which, however, has a feeble power of self-differentiation as a double assurance.

4. This provides a confirmation of previous suggestions that an organ which is vestigial in the sense that it has lost its physiological function may be retained in ontogeny because it still fulfils the morphogenetic function of providing a stimulus essential for the development of other, physiologically more important, structures.

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EXPLANATION OF PLATE I

- Fig. 1. No. 35-022. Embryo of 5 somites operated as in Fig. 2. The pronephros is separated from the lateral plate and joined to the somitic mesoderm.
 Fig. 2. No. 35-029. Embryo of 5 somites at operation; a longitudinal cut was made just lateral to the axis in the posterior region. Cultivated 29 hr. The figure shows lateral plate mesoderm and pronephros, somitic mesoderm is absent, but there may be a small trace of neural tissue. *Pn.* pronephros, *Nt?* possible neural tissue.
 Fig. 3. No. 36-31p. Embryo of 12 somites at operation. A transverse cut was made on the right side posterior to the last somite. Cultivated about 24 hr. No Wolffian duct is present posterior to the cut on the operated side, which is at the left in the figure. *W.D.* Wolffian duct.
 Fig. 4. No. 37-11. Embryo of about 12 somites at operation. Transverse cut as in Fig. 3, embryo then incubated *in ovo* for a further 2 days. No Wolffian duct and no mesonephros on operated side. *W.D.* Wolffian duct, *M.* mesonephros.
 Fig. 5. No. 37-13. Operation as in Fig. 4. Small patch of mesonephric tissue (*M'*) on operated side. Note well-shaped neural tube in absence of notochord.
 Fig. 6. No. F3b-13. *Rana*. The pronephric rudiment was removed from the left side in the young tail-bud. No Wolffian duct or mesonephros present on the operated side, which is to the right in the figure.

Note added to proof. Since this article was sent to press, Grünwald has reported similar experiments which led to the same conclusions (*Arch. EntwMech. Org.* **136**, 786).

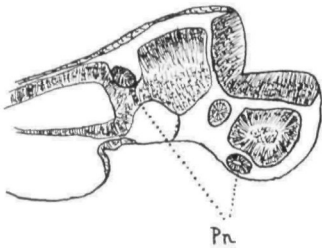


Fig. 1.

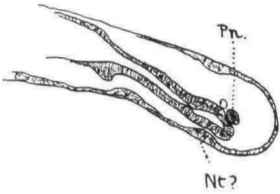


Fig. 2.



Fig. 3.

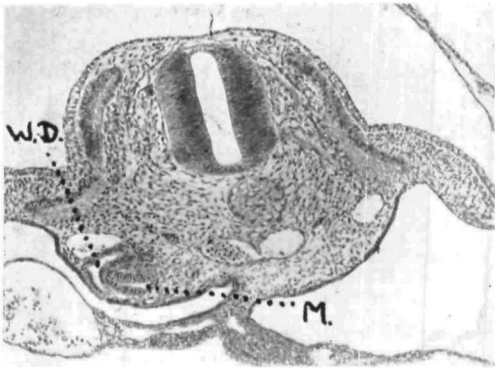


Fig. 4.

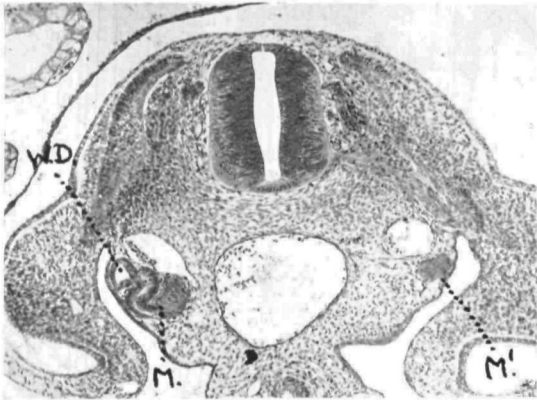


Fig. 5.

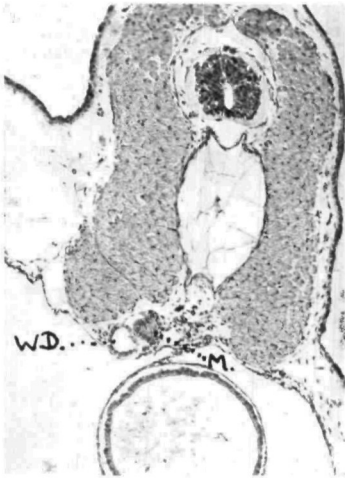


Fig. 6.

WADDINGTON.—THE MORPHOGENETIC FUNCTION OF A VESTIGIAL ORGAN
IN THE CHICK (pp. 371—376).