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The Formation of the Mesoderm in Urodelean Amphibians

V. Its Regional Induction by the Endoderm

E.C. Boterenbrood and P.D. Nieuwkoop

Hubrecht Laboratory, Utrecht, Holland

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Summary. Dorsal (D), lateral (L and R), and ventral (V) portions of the endoderm of blastulae of *Ambystoma mexicanum* of different age (stages 8^+ to 10^-) were combined with ectodermal caps of stage 8^+ blastulae. All V and most L and R portions induced only ventrocaudal mesodermal structures — "ventral" type of mesoderm induction. Almost all D portions induced much more voluminous structures of predominantly axial character — "dorsal" type of mesoderm induction. The difference in mesoderm-inducing capacity of the dorsal as against the lateral and ventral endoderm is probably purely quantitative in character. The dorsal endoderm exhibits a pronounced dominance in mesoderm-inducing capacity. During the early symmetrization of the amphibian egg it is apparently especially the presumptive dorsal endoderm that becomes endowed with strong mesoderm-inducing properties.

A comparison of the results obtained with endodermal portions of blastulae of different age showed that the mesoderm-inducing capacity first begins to decline in the dorsal endoderm (around stage 9), subsequently in the lateral, and finally in the ventral endoderm (at stage 10^-). At stage 10^- the dorsal endoderm no longer has mesoderm-inducing capacities.

In the recombinates there is a striking correspondence between the regional differentiation of the mesoderm and that of the endoderm. The latter differs markedly from the presumptive significance of the various endodermal regions in the normal embryo.

Primordial germ cells, which constitute a characteristic component of the "ventral" type of mesoderm induction, can be induced not only by ventral, but also by lateral and to some extent even by dorsal endoderm. The development of primordial germ cells from the ectodermal component of the various recombinates indicates that in the urodeles the origin of the primordial germ cells differs markedly from that in the anurans.

From experiments in which recombinates were made of animal, ectodermal caps and vegetative, endodermal portions of blastulae of *Ambystoma mexicanum*, Nieuwkoop (1969a) concluded that the mesoderm arises from the ectodermal "half" of the blastula under an inductive influence exerted by the endodermal "half". Sri Sudarwati and Nieuwkoop (1971) demonstrated that the same conclusion holds for the anuran *Xenopus laevis*. The final proof for an exclusively ectodermal origin of the mesoderm was furnished by Nieuwkoop and Ubbels (1972) using xenoplastic recombinates of portions of blastulae of *Ambystoma mexicanum* and *Triturus alpestris*, as well as recombinates of ^{3}H-labelled and unlabelled *Ambystoma* material. The xenoplastic recombinates moreover showed that the inductive action of the endoderm not only led to the formation of the various mesodermal structures, but also to a partial endodermization of the ectoderm, i.e. to the formation of the pharynx endoderm. Endodermization of the ectodermal part of the *Ambystoma mexicanum* blastula can be strongly enhanced by Li when acting upon the uncoated, blastocoelic side of the ectoderm (Nieuwkoop 1970).

Translocation experiments in which the animal, ectodermal "half" of the *Ambystoma mexicanum* blastula was rotated through 0, 90 and 180° with respect to the vegetative yolk mass, demonstrated that the dorso-ventral organization of the induced mesoderm finds its origin in a dorso-ventral polarization of the endoderm, and not in a polarization of the ectodermal "half" of the embryo, notwithstanding the fact that the grey crescent region (the future organization-centre) is partially included in the ectodermal "half" (Nieuwkoop, 1969b). It is particularly the latter observation that calls for a further analysis of the mesoderm-inducing capacity of the endoderm, especially along its dorso-ventral axis. This has been approached by comparing the inductive capacities of dorsal, lateral, and ventral portions of the endoderm when acting upon competent ectodermal "halves". Thus some further insight has been obtained into the character of the dorso-ventral polarization of the amphibian egg during early development (see also Ancel and Vintemberger, 1948; Pasteels, 1964; Nieuwkoop, 1973). Moreover, the temporal course of the induction process was studied by comparing the mesoderm-inducing capacities of the above endodermal regions taken from blastulae of increasing age.

Material and Methods

Blastulae of *Ambystoma mexicanum* were used for the experiments. Embryos were selected which showed a clear dorso-ventral pattern of pigmentation — i.e., lighter pigmentation and a rather vague boundary between the pigmented and unpigmented areas indicating the dorsal side, as against darker pigmentation and a fairly sharp boundary between the two areas on the lateral and ventral sides of the embryo. Moreover, on the dorsal side the embryo shows a tendency to flatten, which is due partly to the further extension of the blastocoelic cavity on the dorsal side, and partly to a more pronounced thinning of the dorsal blastocoelic roof. The identification of the dorso-ventral axis at blastula stages is only approximate, however; the criteria are not completely unambiguous and there is a variation in pigmentation pattern among individual embryos.

Blastulae of stages 8^+, 9 and 10^- (Harrison, 1969) were used, last-named stage being characterized by a long, slightly curved pigment line as the first indication of the future blastoporal groove, the other stages by the number of blastomeres in the animal pole region. The endoderm was isolated by cutting the blastula along the equator and removing the peripheral rim from the vegetative half (see Fig. 1b.—this rim representing the greater part of the presumptive mesoderm as well as some peripheral persumptive endodermal material.) The endoderm—without losing sight of its original dorso-ventral orientation—was cut into four portions, dorsal (D), left (L) and right (R), and ventral (V) (see Fig. 1c). Loose yolk material originating from damaged cells was carefully removed.

Each of the four endodermal portions was recombined with one entire ectodermal cap, which was always taken from blastulae of stage 8^+ by cutting slightly above the equator, more so on the dorsal side, in order to avoid inclusion of already induced mesoderm (see Fig. 1a). The ectoderm soon surrounded the endoderm, so that a nearly spherical "blastula" of reduced size was formed. The great majority of the recombinates later exogastrulated.

Isolation of the endoderm at successive stages meets with the difficulty that the shape of the endodermal mass changes during development, particularly between stages 9 and 10^-. Ingression of superficial endodermal cells as well as events preparatory to blastoporal groove formation lead to a marked reduction of the endodermal area on the vegetative surface of the embryo. The older the blastula, the smaller is the outer surface of the endodermal mass, and the greater its height. Control explants showed that at stage 10^- the blastoporal groove region was often included in the isolated dorsal endodermal portion, whereas this was not the case at stage 9 and earlier. Therefore, at stage 10^- possible contamination of the dorsal endodermal portion with presumptive head meso- and endoderm must be taken into account.

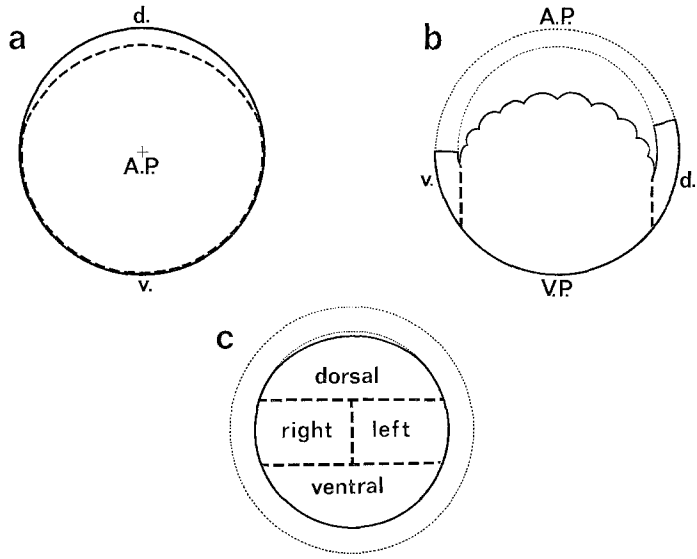

Fig. 1 a—c. Operation diagram. a) Excision of animal, ectodermal cap of blastula along interrupted line. b) Isolation of the vegetative yolk mass by subsequent removal of peripheral equatorial zone. c) Subdivision of the vegetative yolk mass into a dorsal, two lateral (right and left) and a ventral portion, each to be recombined with a whole ectodermal cap. *A. P.* animal pole; *V. P.* vegetative pole; *d* dorsal; *v* ventral

The recombinates were reared in Niu and Twitty solution (with 20000 I.U./L Na penicillin and 100 mg/L streptomycin) at 22° C for 10 days, fixed in Smith's fixative, block stained in borax carmine, embedded in paraffin wax, sectioned at 10 μ, and finally counterstained with aniline blue-Orange G.

The material was analysed by determining the incidence of six different mesodermal structures in each recombinate. Differences in results between recombinates containing D, L, R and V endoderm were tested by applying the sign test (Siegel, 1956, $p \leq 0.05$) to the incidences of the respective mesodermal structures in pairs of "matching" recombinates (i.e., recombinates containing endodermal portions of one and the same blastula). Due to the fact that 10-day-old recombinates show a considerable and varying degree of swelling, no accurate volume measurements of the mesodermal structures could be made.

Three control series were made; in one series the ectodermal cap of stage 8^+, and in another the four endodermal portions isolated at the three stages tested were cultured separately as explants. In the third control series the four endodermal portions isolated at stage 8^+ were fixed immediately after the removal of loose yolk material, and their volume was determined in serial sections.

Results

Control series

In a series of 12 *ectodermal* explants cultured for 10 days no mesodermal structures were formed. Seven cases showed local neural activations consisting of small prosencephalic brain vesicles induced in the ectoderm under the influence of the culture medium (Sala, 1956; Nieuwkoop, 1963).

A series of 60 *endodermal* explants consisting of 5 sets each of D, L, R, and V portions of the endoderm of stages 8^+, 9 and 10^-, respectively, were cultured

for 10–18 days, during which they either lost a large number of cells or disintegrated entirely. Microscopical examination revealed that the explants consisted largely or entirely of undifferentiated endodermal cells. Thirteen out of the 60 explants showed minute mesenchymal cell clumps containing some rudimentary pronephric tubules (8 cases) and/or some muscle cells (3 cases), surrounded by undifferentiated endoderm. These mesodermal differentiations probably arose from small clumps of pigmented ectodermal cells, which are sometimes found lying on top of the endodermal mass when opening the blastula, and which represent cells fallen down from the roof of the blastocoel. They are dificult to remove completely, the more so since in some cases they slip in between the large endodermal cells. A small pharyngeal structure surrounded by some mesenchyme had differentiated in only one D explant of stage 9 and in two D explants of stage 10^-.

Volume measurements of the *endodermal* portions were made on 10 sets each of D, L, R, and V portions of the endoderm of stage 8^+, fixed immediately after isolation. They yielded the following volume ratios D:L:R:V = 10:8:7:12. The differences in volume must be partly due to an initial inequality of the excised masses, and partly to the extrusion of varying numbers of damaged yolk cells, which is most pronounced in the isolated lateral portions.

Series I: Recombinates of Portions of Stage 8^+ Endoderm with Stage 8^+ Ectoderm

This series comprises 11 complete sets of experiments, each set consisting of a D, L, R, and V portion of the endoderm of the same egg, each combined with an ectodermal cap.

Pronounced differences exist between the mesodermal structures induced by the various endodermal portions (see Table 1). The most striking difference is the consistent absence of notochord in V recombinates and of blood cells in D recombinates, while various mesodermal components show pronounced differences in incidence. The availability of complete sets of experiments allows a direct comparison of differentiation in recombinates made with endodermal portions of one and the same blastula. When the incidence of each of the mesodermal components in such related recombinates is compared, D recombinates are found to have a significantly higher incidence of notochord and somite tissue and a significantly lower incidence of primordial germ cells in comparison with L, R, and V recombinates. The incidence of pronephros and Wolffian duct in related D, L, R, and V recombinates does not differ markedly, and neither does the incidence of somites, primordial germ cells, and blood cells in L, R, and V recombinates.

On the basis of the structural composition of the recombinates two types of mesodermal differentiation can be distinguished:

a) A so-called "dorsal" type, characterized on the one hand by the presence of notochord (accompanied by spinal cord, sometimes also by hindbrain and even forebrain[1]) flanked by two rows of somites, and on the other hand by small numbers or complete absence of primordial germ cells and absence of blood cells (see Fig. 2).

1 In cases with forebrain, prechordal mesoderm and sometimes pharynx endoderm were also present, representing the ultimate steps in the vegetalization of the ectoderm (see Nieuwkoop and Ubbels, 1972).

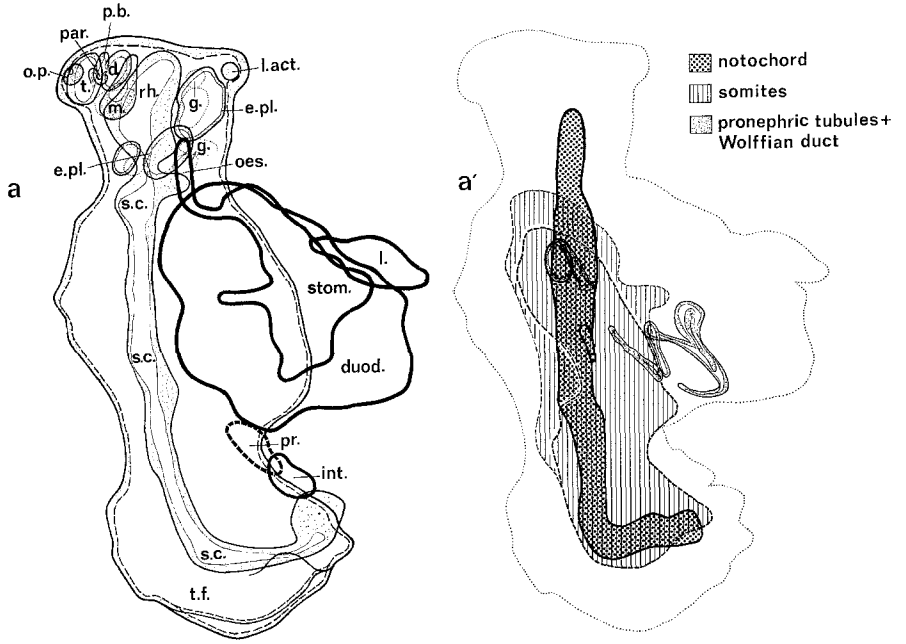

Fig. 2. Graphical reconstruction of a differentiated recombinate involving dorsal endoderm, representing the "dorsal" type of mesoderm induction. Large notochord, two rows of voluminous somites, and rudimentary nephric structures shown separately in figure (a'). The corresponding ecto-neurodermal and endodermal structures are shown in figure (a). *d* diencephalon; *duod.* duodenum; *e.pl.* ear placodes; *g.* ganglia; *int.* intestine; *l.* liver; *l. act.* local activation; *m.* mesencephalon; *oes.* oesophagus; *o.p.* olfactory placode; *par.* paraphysis; *p.b.* pineal body; *pr.* proctodaeum; *rh.* rhombencephalon; *s.c.* spinal cord; *stom.* stomach; *t.* telencephalon; *t.f.* tail fin

b) A so-called "ventral" type, characterized on the one hand by the consistent presence of primordial germ cells, often in large numbers, and on the other hand by the absence of notochord and well-organized somites (in a number of cases a moderate amount of muscle in sheet-like arrangement is formed). In several cases a varying amount of blood cells is present (see Fig. 3). In both the "dorsal" and the "ventral" type pronephros and Wolffian duct are nearly always present in varying amounts.

In this series ten D recombinates belong to the dorsal type and one to the ventral type (the recombinates of the matching L, R, and V endodermal portions of the latter embryo also belong to the ventral type). In the R recombinates the dorsal type is found in only two cases, but the matching L ones show the ventral type. A similar situation is encountered in two L recombinates showing the dorsal type, the matching R ones being of ventral type. The fourteen remaining lateral recombinates all show the ventral type. Finally all the V recombinates are of ventral type. The occurrence of the dorsal type of differentiation in some L and R recombinates, coinciding with the occurrence of the ventral type in the matching reciprocal ones, strongly suggests that notochord dif-

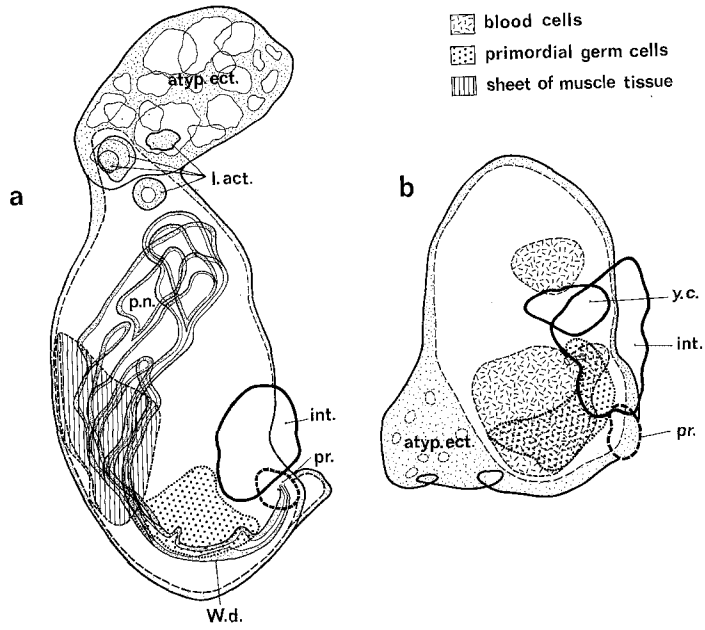

Fig. 3a and b. Graphical reconstructions of two differentiated recombinates involving ventral endoderm, representing two variations of the "ventral" type of mesoderm induction. a) Recombinate with sheet of muscle tissue, voluminous nephric structures, Wolffian duct, and area containing primordial germ cells. b) Ditto, mainly with blood islands and primordial germ cells. *atyp. ect.* atypical ectoderm; *int.* intestine; *l.act.* local activation; *p.n.* pronephric tubules; *pr.* proctodaeum; *W.d.* Wolffian duct; *y.c.* yolk cells

ferentiation in lateral recombinates is due to inaccuracies in the identification of the plane of bilateral symmetry of the embryo resulting in occasional errors in the planes of cutting, and leading to the inclusion of some dorsal endoderm in lateral portions. (Keeping in mind how difficult it is to identify accurately the plane of bilateral symmetry of the embryo, such inaccuracies seem unavoidable.) This obvious interpretation of the occurrence of the dorsal type of differentiation in some lateral recombinates leads to the conclusion that it is particularly *the dorsal endoderm which is endowed with a special mesoderm-inducing capacity*, that for the induction of notochord and accompanying somites.

For a comparison of the amounts of mesoderm in the dorsal and ventral types of induction the total cell mass of all the mesodermal structures formed should be taken into account. Pronephros and Wolffian duct as well as mesenchyme on an average contribute approximately equal amounts in dorsal- and ventral-type inductions, although there may be considerable variations among individual cases. Primordial germ cells and blood cells constitute only a minute proportion. Notochordal cells are strongly swollen in the ten-day-old recombinates, but the actual cell mass of the notochordal structure constitutes only a moderate proportion. It is the somite tissue which, when present, forms by far the largest component. The formation of two rows of compact somites in the dorsal type of mesoderm induction, in contrast to the sheetlike muscle differentiation in the

ventral type, implies that the dorsal inductions are markedly larger than the ventral ones. At a rough estimate the total number of mesodermal cells in inductions of the dorsal type is several times larger than that in recombinates of the ventral type. This observation, taken together with the significantly higher frequency of muscle formation in D than in L, R, and V recombinates, leads to the conclusion that *the inducing capacity of the dorsal endoderm* not only leads to a different type of mesoderm induction, but *is also markedly stronger than that of the lateral and ventral endoderm.* There is no ground for attributing this difference in inductive capacity to relative differences in volume between the inductors, because it is the ventral portion that has the largest average size of all (see p. 322).

Just as in the normal embryo, recombinates of the dorsal type show a distinct regional correspondence between the cranio-caudal pattern of differentiation of the mesoderm on the one hand as reflected in the localization of the head (prechordal plate), anterior trunk (heart and pronephros), posterior trunk (Wolffian duct, primordial germ cells and blood cells) and tail mesoderm, as well as in the gradual reduction of the size of the notochord — and the topography of the various brain parts and the spinal cord on the other hand (see Fig. 2).

The endodermal structures are nearly always abnormal in that their configuration is completely or partially inside-out. In dorsal-type recombinates the differentiation of the pharynx, oesophagus, stomach, liver, pancreas, duodenum, and intestine, as well as that of proctodaeum can nevertheless be distinguished. Their regional topography shows a general correspondence with the cranio-caudal regionality of the mesoderm (see Fig. 2). Recombinates of the ventral type contain only intestine, the most caudal endodermal structure. It is interesting to note that in the only D recombinate showing mesoderm induction of the ventral type the only endodermal differentiation is the intestine.

Series II: Recombinates of Portions of Stage 9 Endoderm with Stage 8⁺ Ectoderm

This series comprises nine complete sets of experiments. The most striking difference with the previous series is that four D and five L recombinates did not form any mesodermal structures at all (see Table 1). This phenomenon will be met again in more pronounced form in series III. It is remarkable that the occurrence of mesoderm induction in L and R recombinates differs significantly (sign test, $p = 0.031$).

The positive cases of the D group as compared with the L, R and V groups of recombinates show some distinct parallels in incidence of the various mesodermal structures with corresponding groups of recombinates of series I. In series II the number of matching recombinates both showing mesoderm formation is, however, too low for statistical analysis. In this series all D recombinates belong to the dorsal type. Three R recombinates are of the dorsal type (although the amount of notochord and somite tissue is rather small), the matching L recombinates being of the ventral type, a phenomenon that was also encountered in series I. The other lateral recombinates as well as all the ventral ones belong to the ventral type.

Table 1. Incidence of induced and self-differentiated (..) mesodermal structures in recombinates of dorsal (D), left (L), right (R), and ventral (V) endodermal portions of blastulae of stages 8^+ (series I), 9 (series II) and 10^- (series III) with ectoderm of stage 8^+, expressed in % of cases showing induced mesodermal structures

Endodermal portions	Total number of cases	Number of positive cases	Notochord (%)	Somites or muscle tissue (%)	Pronephros (%)	Wolffian duct (%)	Primordial germ cells (%)	Blood cells (%)
Stage 8^+ (series I)								
D	11	11	91	100	100	64	46	—
L	11	11	18	55	82	64	82	36
R	11	11	18	46	82	91	91	18
V	11	11	—	46	91	91	100	55
stage 9 (series II)								
D	9	5	100	100	100	100	40	—
L	9	4	—	50	50	50	100	50
R	9	9	33	56	89	100	89	40
V	9	9	—	56	78	78	100	30
stage 10^- (series III)								
D	9	0+(3)	(+)	(+)	—	—	—	—
L	9	1	—	—	+	+	+	+
R	9	1+(1)	(+)	(+)	+	+	+	—
V	9	8	—	13	88	75	100	50

Just as in series I there is again a striking correspondence in cranio-caudal regionality between the differentiated mesodermal, neural, ectodermal, and endodermal structures.

Series III: Recombinates of Portions of Stage 10^- Endoderm with Stage 8^+ Ectoderm

This series comprises nine complete sets of experiments. The number of recombinates without any mesodermal differentiation is much larger than in the previous series: six D, eight L, seven R, and one V (see Table 1). When the three positive cases among the D recombinates are studied in detail it is evident that they contain *only anterior* mesodermal and endodermal differentiations, such as prechordal mesoderm, anterior notochord with adjacent somites, pharynx endoderm, and anterior brain parts (sometimes accompanied by hindbrain). There is no typical truncal axis formation, and more caudal differentiations, such as pronephros, Wolffian duct, duodenum, intestine, and spinal cord are completely lacking. The latter phenomenon is not seen in the two previous series. As already mentioned (page 320) the D portion isolated at stage 10^- often includes the region of the future blastoporal groove. The observed cranial meso- and endo-

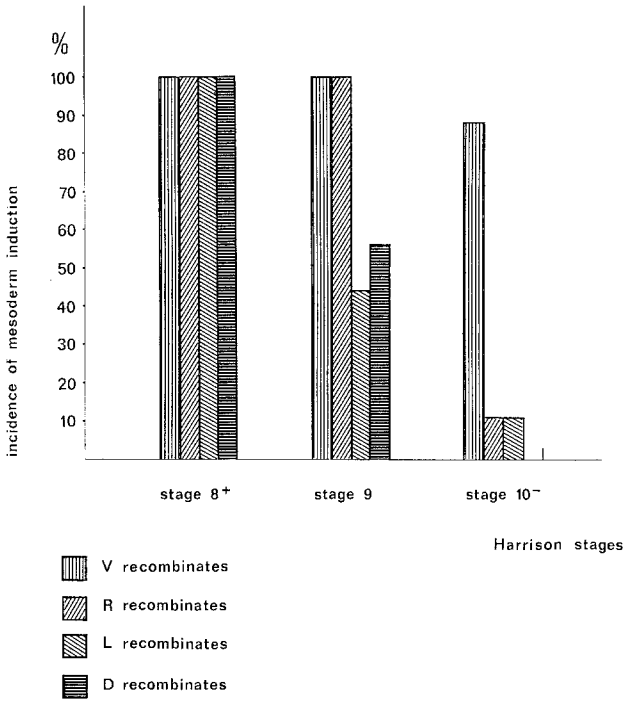

Fig. 4. Incidence of mesoderm induction in recombinates with ventral (*V*), right (*R*) left (*L*), and dorsal (*D*) endoderm respectively, taken from successive stages of development (8^+ to 10^-) and recombined with ectodermal caps of stage 8^+

dermal structures have therefore most likely arisen from the isolated D portion itself as a result of induction by the dorsal endoderm before isolation. Consequently, all the D recombinates may be considered negative as far as *de novo* mesoderm induction is concerned. One of the R recombinates shows cephalic structures similar to those found in the three D recombinates. Only one R and one L recombinate show induced mesodermal structures, whereas nearly all V recombinates contain induced mesoderm. The induced mesodermal differentiations in the L, R, and V recombinates all belong to the ventral type, but muscle differentiation is nearly always absent and the primordial germ cells are markedly atypical in six out of the ten cases, large bilobed nuclei being present only rarely.

From these results it may be concluded that at stage 10^- the ventral endoderm still possesses almost normal inductive capacity, whereas the dorsal endoderm has lost this capacity completely, and the lateral endoderm almost completely. Comparing the results of the three series (see Fig. 4) it may therefore be concluded that a *decline in inductive capacity of the endoderm begins at stage 9, starting on the dorsal side and proceeding towards the ventral side, where it does not become manifest until stage 10⁻.*

It may finally be mentioned that in those recombinates of series III in which no mesodermal structures were induced, the endoderm nevertheless showed some

weak tendencies towards differentiation of pharynx, stomach and liver in dorsal recombinates, and of liver, pancreas and intestine in lateral recombinates. Although this self-differentiation of the endoderm in mesoderm-free recombinates is highly atypical, it indicates that at stage 10⁻ the endoderm possesses weak regional differentiation tendencies, which, however, cannot be properly expressed in the absence of mesoderm. The fact that such self-differentiation does not take place in the endodermal control explants is probably due to the absence of the ectoderm, which in the recombinates forms a protective cover.

Discussion

1. The Regional Induction of the Mesoderm

The experiments described above demonstrate that regional differences in mesoderm-inducing capacity exist within the endoderm. It is particularly the dorsal endoderm which differs in inductive capacity from the other regions. It does not only show *a much higher mesoderm-inducing capacity*, but also *a different type of induction*—notochord and somites, in combination with pronephros and Wolffian duct, are predominantly induced, whereas the inductions by lateral and ventral endoderm are characterized by primordial germ cells and blood cells in combination with Wolffian duct, pronephros, and muscle tissue.

Two questions arise as to this special mesoderm-inducing capacity of the dorsal endoderm: 1) is the observed difference of a *quantitative* nature only or does it also involve a *qualitatively* different type of induction, and 2) what is the role of the dorsal endoderm in normal regional mesoderm formation?

The differences between the "dorsal" and the "ventral" type of mesodermal induction are partly absolute, partly relative in character. The absolute differences concern the presence or absence of notochord and blood cells. The relative differences are expressed in differences in incidence and amount of somite tissue and primordial germ cells. Finally, the differentiation of pronephros and Wolffian duct hardly differs among the two types of mesoderm induction. These observations plead in favour of a *quantitative difference in inductive capacity*. The one case in which a dorsal endodermal portion elicited a typical ventral type of mesoderm induction gives additional evidence. This suggestion is moreover supported by data from the literature. Here we will only mention Yamada's (1950) experiments in which ventral mesoderm of neurula stages could form dorsal mesodermal structures under the influence of brief ammonia treatment (for further evidence, see Nieuwkoop, 1973).

When comparing the inductive capacities of the *isolated* dorsal, lateral, and ventral regions of the endoderm with what might have been expected according to the anlage map of the marginal zone of the intact urodelan blastula (Fig. 5), it becomes evident that there are marked discrepancies as far as the lateral endoderm is concerned. In normal development a large part of the somite region as well as the lateral horns of the notochordal anlage develop from the marginal zone region adjacent to the lateral endoderm. The isolated lateral endoderm, however, induces mesoderm of ventral type. It must therefore be concluded that upon isolation the lateral endoderm undergoes a considerable drop in inductive capacity, which becomes virtually equal to that of the isolated

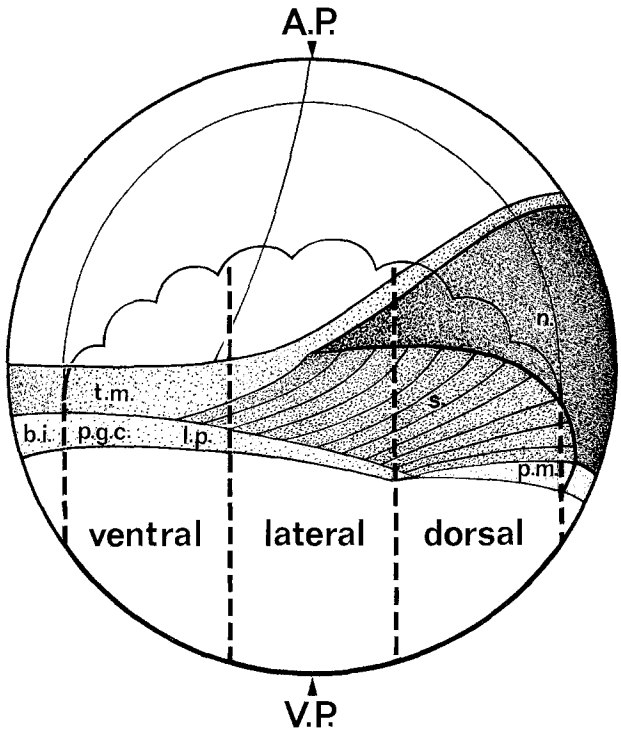

Fig. 5. Projection of the various portions of the vegetative yolk mass, tested separately for mesoderm-inducing capacity, upon the analage map of the presumptive mesoderm at the middle blastula stage (map after Vogt, 1929). *A.P.* animal pole; *b.i.* blood island; *l.p.* lateral plate; *n.* notochord; *p.g.c.* primordial germ cells; *p.m.* prechordal mesoderm; *s.* somites; *t.m.* tail mesoderm; *V.P.* vegetative pole

ventral endoderm. Conversely, *in situ* the mesoderm-inducing capacity of the lateral mesoderm must therefore be markedly enhanced by the presence of the dorsal endoderm, whose own inductive capacity apparently does not change upon isolation. This leads to the conclusion that the dorsal endoderm exhibits a clear *dominance* in the process of mesoderm induction. This means that the dorso-ventral organization of the embryo—expressed in dorsal axis formation in the mesodermal mantle after gastrulation—is chiefly due to the strong inductive capacity of the dorsal endoderm. This conclusion throws some new light on the origin of dorso-ventral polarization in the amphibian egg. Whatever may be the role of the grey crescent in the determination of dorso-ventral polarity (see Nieuwkoop, 1969b, p. 312) it is apparently especially the *dorsal* presumptive endoderm that becomes endowed with strong mesoderm-inducing properties during the symmetrization of the egg (see also Nieuwkoop, 1973).

When comparing the inductive capacities of the various isolated regions of the endoderm at the three successive stages tested, it is evident that the inductive capacity first begins to decline in the dorsal endoderm (around stage 9), subsequently in the lateral endoderm (probably only slightly beyond stage 9) and

finally, about four hours later, in the ventral endoderm (stage 10^-) (see Fig. 4). In contrast to the gradual progression of the decline in the ventral direction, the actual effect of the decline clearly has the character of an all-or-none response: either the induction is normal or there is no induction at all. This may indicate that the decline is due to a process which is superimposed upon, and interferes with, the process of mesoderm induction as such.

The present experiments show that at stage 9 the incidence of mesoderm induction in L recombinates is much lower than in R recombinates. This would mean that on the left side the decline is advanced with respect to the right side. Such a relatively more advanced mesoderm induction on the left side may be related to the more advanced determination of the left heart anlage which was observed by various authors and leads to the asymmetry in shape and position of the normal heart (see von Kraft, 1971). The corresponding asymmetry in the disposition of the alimentary canal may be due to a secondary influence of the mesoderm on the differentiation of the endoderm (see below).

2. The State of Determination of the Endoderm at the Blastula Stage

Nieuwkoop and Ubbels (1972) have demonstrated that in xenoplastic recombinates involving *Ambystoma mexicanum* and *Triturus alpestris* stage 8^+ blastulae, as well as in recombinates of ^{3}H-labelled and unlabelled material of blastulae of the same stage, the vegetative material only gives rise to endodermal structures, whereas the animal cap material is pluripotent. The present experiments show that mesodermal inductions of ventral type induced by V, L, R, and in one case even by D endoderm were always accompanied by intestine as the only endodermal differentiation, whereas mesodermal inductions of dorsal type induced by the D endoderm (presumptive anterior endoderm) were accompanied by a whole range of endodermal structures comprising all cranio-caudal levels. It must therefore be concluded that in general the endoderm differentiates in accordance with the type of mesoderm formed in the recombinates, and not according to its presumptive significance.

At stage 8^+ the dorso-ventral polarization of the endoderm therefore only expresses itself in its mesoderm-inducing capacity, but the endoderm is not yet programmed for regional differentiation. In normal development this programming, which apparently occurs under the influence of the induced mesoderm, probably does not take place until a late blastula stage, as indicated by the beginning tendency for self-differentiation of the endoderm in the dorsal and lateral recombinates of series III (p. 328) (see also Holtfreter, 1938; Nieuwkoop, 1973).

3. The Origin and Differentiation of the Primordial Germ Cells in Urodeles

In all mesoderm inductions of ventral type large numbers of primordial germ cells were found. They were even observed in some inductions of the dorsal type where some ventro-caudal mesoderm was formed. Like the blood cells, the

primordial germ cells apparently constitute a characteristic element of the ventro-caudal mesoderm.

The primordial germ cells were frequently found in close topographical relationship with caudal Wolffian duct and proctodaeum, although a strict spatial correlation with any of these structures could not be demonstrated. They always lay below or between the endothelial linings of the coelomic cavities. Although in normal development the primordial germ cells originate from the ventral and latero-ventral mesoderm (see Nieuwkoop, 1947)—which borders on the ventral endoderm—they can apparently just as well be induced by lateral and even by dorsal endoderm. Whether they arise from special, "predetermined" cells or from "common" cells is still an open question. Germ cell formation in urodeles seems to be in sharp contrast to the strict endodermal germ cell lineage in anurans (see Bounoure, 1939). Further analysis of the origin of the primordial germ cells in urodeles is in progress.

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Dr. E.C. Boterenbrood
Hubrecht Laboratory
Utrecht
Holland