Bone-marrow of the Guinea-pig as a Mesodermal Inductor in Implantation Experiments with Embryos of *Triturus*

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WITH ONE PLATE

INTRODUCTION

Levander (1938) has shown that an alcohol extract prepared from bone can, when injected into rabbit muscle, induce there cartilage and bone cells. This observation has been confirmed by several investigators (Annersten, 1940; Bertelsen, 1944; Lacroix, 1945; Levander, 1949; Willestaedt, Levander & Hult, 1950). Of these, Bertelsen has shown in addition that not only is an extract prepared from bone proper active, but also that an extract prepared from bone-marrow is even more active. In addition to the authors mentioned above, Schreiber (1950) has lately dealt with the problem. He has assumed, on good grounds, that the agent inducing bone and cartilaginous tissue in the experiments mentioned above is similar, as regards its chemical nature, to the 'spinal' inducing agent in my experiments (Toivonen, 1940, 1949 a and b, 1950). This assumption of Schreiber's has prompted me to investigate experimentally the inducing action of bone-marrow when implanted into the gastrula of *Triturus*.

MATERIAL AND METHODS

Gastrulae of *Triturus vulgaris* were used as the host in all the experiments. The inductor material was marrow from the thigh-bone of the guinea-pig, which had been immersed in 70 per cent. alcohol for approximately 24 hours. Operations were carried out by the implantation method, using Holtfreter saline with sodium sulpha-diazine added as a bactericidal agent. Embryos were cultured usually for 2 weeks. Specimens were fixed in Bouin's fluid and first stained in bulk with borax-carmine; the sections were stained further with Picroblau-schwarz.

RESULTS

On external examination of the experimental animals the structures induced by the bone-marrow appeared very similar to those caused by alcohol-treated

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kidney tissue in my earlier experiments (1940, 1949 a and b, 1950). In those of the experimental animals in which induced structures could be seen externally, tail-like appendages were common (40 per cent.), and an irregular fin was found in many others (24 per cent.). The inductor thus seemed to have a 'spinal' inducing action.

Altogether induction occurred in thirty animals. Microscopic preparations showed that in five animals the extra formation was in continuity with the central nervous system of the host. In all these cases a very complete extra tail was found, containing, in addition to the notochord and rows of paired myotomes, a spinal cord. Since in these specimens the connexion between the induced structure and the host was so direct, they will not be discussed here (cf. Holtfreter, 1936, p. 532; Toivonen, 1940, p. 19).

An analysis was made of the frequency of the various types of differentiated structures induced in the remaining twenty-five animals. The results are seen in Text-fig. 1.

A scrutiny of the preparations reveals the surprising fact that no actual neural tissue was ever induced. Not even unspecialized neural vesicles are found in the induced structures. In some of those animals (32 per cent.) in which a tail-like formation was externally visible the mesenchyme, containing numerous melanophores, also contained isolated, more darkly staining cells, which might perhaps be regarded as neural cells. It has not, however, been possible to confirm the neural nature of these cells, and the presence of neural cells is therefore still to be regarded as questionable.

The weakest level of mesodermal induction is represented by increased mesenchyme in the host at the site of the implant. This mesenchyme usually contained numerous melanophores. In nine cases (36 per cent.) one or two extra forelimb rudiments had been produced (Plate, figs. B and C). In many cases large blood lacunae (Plate, fig. A) were found in connexion with the induced structures. These have not been taken into account in the analysis, since there is no means of discovering whether they were induced or were caused mechanically by the implant. Myotomes (Plate, figs. A and B) and notochord (Plate, fig. C) occur relatively seldom in the inductions (32 and 16 per cent.). On the other hand, a typical pronephros (Plate, fig. C) with its Wolffian ducts was common (68
per cent.). If an extra proctodaeum with its anal opening was found in the animal, the paired induced Wolffian ducts usually opened into it. An extra anal opening and proctodaeum (Plate, fig. D) occurred in 64 per cent. of the twenty-five experimental animals included in the analysis. The proctodaeum was in most cases connected with the intestine of the host, although the connexion was usually extremely narrow and in my opinion secondary. In three cases anus and proctodaeum were quite independent of the host intestine. It is worth noting that the extra anal opening can occur even at the level of the anterior portion of the liver; in most cases, however, it was found in the hind part of the trunk.

**DISCUSSION**

The inducing action of alcohol-treated bone-marrow is to be regarded as solely mesodermal, except for some possibly neural cells. For the ectodermal proctodaeans were, in my opinion, secondarily evoked by the induced mesoderm (cf. Holtfreter, 1936, p. 521), and not directly caused by the inductor. Alcohol-treated bone-marrow is thus a very specific mesoderm-inductor. In this respect it is more specific in its action than any killed inductor previously used. For instance, the alcohol-treated kidney tissue of the guinea-pig, used in my earlier experiments (Toivonen, 1940, 1949 a and b, 1950), is less specific in its action, since in addition to mesoderm it also regularly induces spinal cord and parts of deutereencephalon together with ear vesicles. Extraction of this kidney tissue in petroleum ether changes its action so that the neural component in the induction is weakened, parts of the brain as a rule do not appear, and the spinal cord is thinner; and in certain individual cases no more than an isolated myotome has occurred, without any neural structures. Extraction in petroleum ether thus changes the inducing action of kidney tissue of the guinea-pig in the direction of mesoderm-induction alone; and it can be assumed that if the extraction could be done very thoroughly, the induction might be similar to that obtained in this investigation with alcohol-treated bone-marrow. The experiments in the present investigation in my opinion show unequivocally that the mesoderm-inducing agent is entirely independent of the agents inducing neural structures. The spinal agent earlier postulated can thus be further divided into components inducing mesoderm and inducing neural structures.

The question now naturally arises as to what is the specific action, independent of the mesoderm-inducing agent, of the neural inductor component contained in the 'spinal' inductor. My earlier experiments with kidney tissue of the guinea-pig, which showed a weak power of archencephalic induction after heat treatment, would seem to indicate that it is archencephalic. We would then be faced with a system of two opposing gradients of agents, which Lehmann (1950, p. 144) has suggested as a possibility: much archencephalic + little mesodermal = archencephalic action; moderate archencephalic + moderate mesodermal = deutereencephalic action; little archencephalic + much mesodermal = spinal action. Kuusi (1951, p. 87), too, regards such a system as probable. I have since,
however, shown (Toivonen, 1951, 1952), when comparing the structures induced by liver tissue of starved and of well-fed guinea-pigs, that during starvation the action of the liver changes in an archencephalic direction; the deuterencephalic component contained in the liver of a well-fed guinea-pig then disappears almost entirely. As an explanation for this phenomenon I regarded it as probable that during starvation a deuterencephalic agent of large molecular size splits into an archencephalic one of small molecular size. Both would thus be rather closely related chemically, but in fact different substances. Since the same number of spinal structures was induced by the liver of both starved and well-fed guinea-pigs, it is evident that the change which occurs does not affect the spinal agent contained in the liver.

It is noteworthy, however, that in no case has liver tissue of either well-fed or starved guinea-pigs induced a typical tail containing a spinal cord. In my opinion the interpretation of this fact must be that the inductor has not contained enough of the mesoderm-inducing agent necessary for this differentiation. For it is possible that the production of the spinal cord is actually brought about by the autonomous growth of the mesoderm, especially the myotomes, which, whilst the tail-bud is developing, at the same time stretches the induced neural structure into the spinal cord.

The present experiments with bone-marrow in any case exclude the possibility that the spinal cord and the neural component in the extra tails can have been secondarily induced by the mesoderm. The ectoderm is apparently by this time already so 'aged' that its competence no longer allows the production of an induced structure of this kind. The prerequisite for the production of a complete tail is thus that the inductor itself should contain adequate amounts of both the mesodermal and the neural agent.

My earlier assumption mentioned above that the neural-inducing agent would prove further divisible into two different components, archencephalic and deuterencephalic, was then advanced as a working hypothesis, and it has not yet been possible to confirm it. If it is true, the deuterencephalic agent contained in the inductor would in my opinion be primarily responsible for the spinal cord produced in connexion with tail formation. Such an assumption is justified by the regional sequence of these structures in the normal embryo.

As regards the opinion that deuterencephalic induction also requires the mesoderm-inducing agent for its occurrence (cf. p. 99), I do not regard it as necessary. Actual direct evidence for this opinion of mine I am not able to present. Indirectly it is supported by experimental series in which no ordinary mesodermal structures occur among the archencephalic and deuterencephalic structures induced. In my earlier material I have discovered isolated cases of this kind. Of the inductors used by Kuusi (1951), the following, for instance, have had such an action: different nuclear fractions of the liver and kidney of the guinea-pig (p. 24, Fig. 9), certain nucleoprotein and plasma protein fractions (p. 33, Fig. 30), and the large granule fractions prepared from the liver of the
same animal (p. 47, Fig. 54). If these inducers were to contain the mesoderm-inducing agent taking part in the induction of deuterencephalic structures, then this agent ought in some specimens to manifest its own effect and at least produce a weak mesodermal reaction. Since this has not happened, the participation of the mesoderm-inducing agent in the induction of deuterencephalic structures is in my opinion very questionable.

This view is more directly supported by the experimental series of Chuang (1939, 1940), in which he investigated the effect of boiling of different duration on the nature of the structures induced by kidney tissue of the mouse and by liver tissue of the newt. In the structures induced by the former no mesodermal material occurred if boiling had lasted over 5 minutes; from the latter, the ability to induce mesoderm had already disappeared in 2 seconds. On the other hand, even after boiling for 1 hour mouse kidney tissue induced parts of the brain and even ear vesicles, and with liver tissue of the newt the frequency of induced ear vesicles and parts of the brain even increased after the ability to induce mesoderm had been lost. Chuang does not analyse the nature of the parts of the brain induced, but since ear vesicles can be regarded as indicators of the deuterencephalic region, their occurrence is evidence of deuterencephalic differentiation in the inductions. But it cannot be thought that the thermolabile mesoderm-inducing agent could survive such a prolonged boiling treatment. Is it not more probable that deuterencephalic induction had occurred without any mesoderm-inducing agent in the inductor?

It was Chuang who first showed conclusively the thermolability of the mesoderm-inducing agent. Later this has been confirmed at least by me and by Okada (1948) and Rotman (Toivonen, 1950, p. 52). Okada's experimental results are interesting because the inductor used by him, the skin of the abdomen of Rana pyrrhogaster, when explanted fresh to an ectodermal vesicle of Triturus induced a purely mesodermal structure similar to that induced by the alcohol-treated bone-marrow used in the present investigation. When Okada further treated pieces of skin for some minutes in boiling water before implantation, only neural and epidermal structures were induced; these structures were, in my opinion, mainly archencephalic. Okada interprets his experimental result by adopting the possibility suggested by Chuang (1939) that the mesoderm-inducing agent changes during heat treatment into a neural-inducing agent. In my opinion this interpretation is hardly likely to be correct. In this case the mesoderm-inducing agent would seem to be destroyed and the neural agent at the same time liberated. It can be regarded as probable that from a fresh piece of skin only mesoderm-inducing agent can be liberated because it occurs in the corium of the ventral skin. After heat treatment those agents can also be liberated which until then have been situated within the poorly permeable epidermal cells, and after the destruction of the mesoderm-inducing agent these may cause a neural induction. It would be interesting to study what would be the effect of treating the fresh skin with alcohol before implantation. The mesoderm-inducing agent
would not then be destroyed, but the original restriction of the release of the substances caused by the poor permeability of the living cells would be eliminated. I would expect that both mesodermal and neural elements would then be induced. Thus in my opinion the mesoderm-inducing agent does not change into the neural one.

Waddington (1952) has recently referred to the experiments of Barth & Graff (1943), and other corresponding experiments, in which the inductor has in one way or another been killed and then explanted into ectodermal vesicles. In these experiments the regionally specific action of the inductor has for the most part been eliminated and above all no mesoderm was induced. Referring to my earlier schematic picture (Toivonen, 1940, p. 135, Fig. 45) I must emphasize that the natural inductor consists of embryonic tissue, in which no great regional differences in the concentrations of substances in the cells can prevail, since no differentiation has yet taken place. When alive, the different regions of the archenteron roof are specific in their action in my opinion mainly because the permeability of the cells is different in different regions, and this regulates the passage of active substances in a way characteristic of each region. When the tissue is killed in one way or another, all the substances contained in it are liberated to compete for the reactive material. Those of the active substances which are overwhelming in amount, or are first able to determine the reactive material, determine the nature of the structures induced. It is evident that the mesoderm-inducing agent in a killed natural inductor is always defeated in this competition when used in explantation experiments. In Waddington’s (1952) latest explantation experiments, in which he has used rather old donors and in addition destroyed the mesoderm-inducing agent with hot water, it appears that the differentiation of the concentration of the neural agents in the different regions has already started. The induction of brain or eye was not uncommon when anterior inductors were used, but was not seen at all with posterior ones. In highly differentiated tissues of adult animals, such as bone-marrow, liver, and kidney, this chemical differentiation has advanced so far that the tissues, even if killed in alcohol, are more or less specific in their action when used as heterogenous inductors. In my opinion, therefore, the inability of the alcohol-killed natural inductor to induce any but neural structures in explantation experiments is only due to the fact that the neural agents camouflage the mesoderm-inducing agent contained in the inductor. I assume that the mesoderm-inducing agent would exert its effect if the neural agents of the inductor were diminished, for instance by extraction with petroleum ether.

SUMMARY

1. Marrow from the thigh-bone of the guinea-pig, treated in 70 per cent. alcohol, has been used as the inductor material in implantation experiments with gastrulae of Triturus.
2. The inductor has induced mesodermal structures such as limb rudiments,
notochords, pronephric tubules, and mesenchyme, and, in addition to these, proctodaeae with anal openings. These last are regarded as secondary induction products of the induced mesoderm. The only suggestion of neural induction consisted of darkly staining cells, which may perhaps be regarded as neural.

3. The results show that the mesoderm-inducing agent is independent of the agents inducing neural structures, and the 'spinal' agent earlier postulated can thus be further divided into components inducing mesoderm and inducing neural structures.

4. The question as to what is the specific action, independent of the mesoderm-inducing agent, of the neural-inducing component in the previously established 'spinal' inductor, is discussed.

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REFERENCES


EXPLANATION OF PLATE

**Fig. A.** Section through an embryo with myotomes and blood-cells induced ventrally.

**Fig. B.** Section through the liver region of an embryo with an induced forelimb rudiment and myotomes.

**Fig. C.** Section through an embryo in which two forelimb rudiments, a notochord, and pronephric tubules have been induced.

**Fig. D.** Section through an embryo with an induced ectodermal proctodaeum.
blood cells
myotome
forelimb rudiment
implant
notochord
forelimb rudiments
pronephros
implant
proctodaeum