Introduction

Since Schreiber (1950) has assumed that the agent inducing bone and cartilaginous tissue in the experiments of Levander and his collaborators (Levander, 1938; Annersten, 1940; Bertelsen, 1944; Lacroix, 1945; Levander, 1949; Willestaedt, Levander, & Hult, 1950) is similar, in regard to its chemical nature, to the spinal inducing agent in my experiments (Toivonen, 1940, 1949a, 1950), I implanted alcohol-treated bone-marrow of the guinea-pig into the gastrulae of Triturus. The results of these experiments, which I have reported earlier in this journal (Toivonen, 1953), showed that the inducing action of this inductor is to be regarded as only mesodermal. Hence the spinal agent earlier postulated can be subdivided into a component inducing neural structures and a component inducing mesoderm, of which only the mesoderm-inducing agent is included in the bone-marrow tissue.

According to Chuang’s (1939, 1940) experimental results—later confirmed by Okada (1940), by Ritumae (Toivonen, 1950, p. 51), and by myself (1953)—the mesoderm-inducing activity is abolished by heating the inductor. Last spring, therefore, I continued the experiments with alcohol-denatured bone-marrow, treating it for 10 minutes in hot water at 80°–90°C before using it as inductor in implantation experiments.

When using whole gastrulae in such experiments there is reason to believe that the fields of the host may have an effect of their own on the nature of the structures induced. At the end of the last operating season, therefore, I also made two series of control experiments using the sandwich method, with inductors either of alcohol-treated bone-marrow, or of the same tissue after treatment in hot water.

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MATERIAL AND METHODS

Gastrulae of Triturus vulgaris were used as the reaction material in all the experiments. The implantations were carried out using Holtfreter saline with sodium sulfadiazine added as a bactericidal agent. In implantations the saline was buffered with phosphates (Deuchar, 1953), which proved to be very suitable. Embryos and explants were usually cultured for 2 weeks. Specimens were fixed in Bouin’s fluid and first stained in bulk with borax-carmine; the sections were further stained with Picroblau-schwarz.

RESULTS

1. Implantation experiments

Alcohol-treated bone-marrow after heat treatment as inductor. The series comprises altogether 33 cases. A detailed analysis of the structures induced is given in Text-fig. 1. It will be seen that, as was to be expected, the previous mesoderm-inducing activity of the alcohol-treated bone-marrow has now entirely disappeared, and the inductor has now induced epidermal differentiations only,

such as balancers, lenses, and lentoids, i.e. it has had a weak archencephalic action. In the majority of the experimental animals the inductor has exerted no perceptible action at all or the reaction of the host has been merely an atypical epidermal proliferation. In one case more darkly staining cells were found, which might perhaps be regarded as nerve cells. In Plate 1 (figs. A-D) are to be seen some examples of the differentiations which have been found in this series.

2. Explantation experiments

A. Alcohol-treated bone-marrow as inductor. This series comprises a few experiments only, but the structures differentiated resemble each other in all the explants which were cultured long enough, so that it is possible to draw some conclusions.

On external examination of the living explants, tail-like formations, limb rudiments, and melanophores were to be seen.

Microscopical preparations showed that in this series all the differentiations were very similar to those that occurred in the corresponding series with whole...
Extramembranous mesenchyme was to be seen in the fins induced. The mesenchyme often also contained melanophores. There were also to be found structures which in normogenesis would develop from the endo-
mesoderm, such as myotomes, notochord, pronephric tubules, and forelimb rudiments in varying ratios. A proctodaeum with anal opening has also been noticed. The similarity to the corresponding series with whole gastrulae is astonishing.

As an example of this series, one explant will be illustrated which contains all the characteristic structures induced in the whole series (Plate 2, figs. A–C); all the differentiations which occurred in the corresponding series with the whole gastrulae are present, and nothing additional to them, neither neural structures nor epidermal differentiations with the exception of the normal epidermis and the proctodaeum.

If the last-mentioned formation is to be regarded as secondarily induced by the induced mesoderm, as I have postulated earlier (Toivonen, 1953), all the induced structures are manifestations of the mesoderm-inducing activity of the inductor. I also wish to lay stress on the fact that this activity has been a very strong one, because there are also present notochord and myotomes, and because the whole explant is differentiated, no part of it having remained as undetermined atypical epidermis.

B. Alcohol-treated bone-marrow after heat treatment as inductor. This series, too, only contains a few cases. But in my opinion they present a very good picture of the activity of this inductor.

External examination of the living explants in itself indicated that the activity of the inductor was very much weaker than in the previous series. Only in a part of the explant was differentiation always determined, the rest remaining as atypical loose epidermis where cell proliferation continued. Externally definable structures such as appeared in the previous series were not to be seen, nor were there melanophores.

Microscopic examination confirmed the fact that the activity of the inductor was very weak. Only in the immediate neighborhood of the inductor was normal epidermis to be observed; elsewhere the epidermis was abnormal. Moreover, the lentoids were a true epidermal differentiation. In some cases more or less abnormal and doubtful balancers were also formed. No signs of mesodermal structures were to be observed.

As an example of the differentiations of the series, a photograph of a section through a typical case is to be seen in Plate 1, fig. E.

DISCUSSION

The results of the experiments reported above confirm my early postulation (Toivonen, 1953) that the agent inducing mesoderm is independent of the agent inducing neural structures. Nor can it be thought, in my opinion, that the meso-
derm-inducing agent is changed by the heat treatment into an agent inducing
neural structures, as was assumed by Chuang (1939, 1940), whose view was also adopted by Okada (1948). In my experiments the effect of the inductor was too weak after heat treatment to allow one to suppose that the previously mentioned mesodermal agent was responsible. In my opinion, the correct interpretation of the results of the experiments is that the mesodermal agent occurring in both the series with alcohol-treated bone-marrow as inductor is capable of affecting in different ways the reactive material in the inductor, so that it was not able to express its effect in the reactive material. When heat treatment has rendered the mesodermal agent inactive, these other agents are enabled to exert their effect.

There is reason to stress that the mesoderm induced by the bone-marrow is derived in normogenesis from two different sources: firstly, it may be axial mesoderm, i.e. endomesoderm derived from the mesoblast proper; and secondly, it may be ectomesoderm, i.e. mesenchyme derived from the neural crest. Thus the explantation experiments show that, as compared with normogenesis, bone-marrow induces the axial mesoderm from foreign tissue, i.e. ectoderm, whilst it induces ectomesenchymal elements from mesoderm as in normogenesis.

As regards the chemical nature of the mesodermal agent, naturally only hypotheses can be put forward. What has previously been presumed to be characteristic of the 'spinal' agent evidently applies to it, however. It is of large molecular size, denatured and inactivated even after a short heat treatment, is soluble in petroleum ether, and, as has recently been shown by Englander, Johnen, & Vahs (1953), it is also inactivated by prolonged alcohol treatment. All these facts indicate that the mesoderm-inducing agent is a thermolabile protein, as postulated earlier for the 'spinal' agent (Lehmann, 1945; Toivonen, 1949 and b, 1950).

As mentioned above, my experiments with bone-marrow were prompted by those of Levander and his collaborators, who found that bone-marrow extracts injected into the cutaneous nerves of the rabbit caused there the production of bone and cartilage cells. It has been assumed that bone regeneration is regulated by an agent liberated from the bone after fracture. This agent affects the reactive cells and causes them to develop into bone cells. The opinion is also expressed that the transplantation of bone to the transplant itself is cytolysed and a regenerative blastema is produced from the neighbouring tissue. In Levander's opinion (1941, 1951) the transplanted epidermis would be cytolysed, but an agent is liberated from the cells, which promotes regeneration of epidermis. Tar and his collaborators have prepared extracts from the epidermis of the rat, which, when injected into the coelom of the rat, have increased mitotic activity. As a result of prolonged injections they have in certain cases been able to produce tumours (Tar, Voutilainen, Kiljunen, & Ravanti, 1953). These examples indicate that in differentiated tissues of adults agents are present which
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are tissue-specific, maintaining and regulating the normal growth and regeneration of the tissues.

These experiments of mine with bone-marrow lend further support to Levan- 
der's (1945) and Schreiber's (1950) view that the tissue-specific agents of the adult 
may possibly be the very same agents that cause induced structures when brought 
in contact with ectoderm capable of differentiation. In this way the problem of the 
substances active in the regulation of embryonic development in the verte-
brates would be one with the problem of the substances taking part in the regula-
tion of the regeneration process, and the investigation of the problem would 
gain a further impetus, since something is already known about each separately.

SUMMARY

1. In previous implantation experiments (Toivonen, 1953) the alcohol-treated 
   bone-marrow of the guinea-pig had proved to be a very specific inductor of meso-
dermal structures.

2. The same material has been treated in addition for 10 minutes in hot water 
at 80°-90° C, and then used as inductor in implantation experiments with whole 
gastrulae of Triturus vulgaris. The results of this experiment show that the pre-
vious mesodermal activity of the inductor has completely disappeared; it now 
induces purely epidermal formations, such as lenses, lentoids, and balancers.

3. In explantation experiments with the 'sandwich' method the alcohol-treated 
   bone-marrow tissue has induced the same mesodermal structures as in the 
   previous series with whole gastrulae.

4. Heat-treated bone-marrow has induced in the 'sandwiches' normal epider-
   mis in the neighbourhood of the inductor, and in addition lentoids and balancer-
   like formations.

5. Because the previously very strong mesodermal activity of the inductor has 
disappeared as a result of the heat treatment and the treated inductor has only 
been able to exert a weak epidermal activity, it is assumed that the heat treat-
ment has inactivated the independent, thermolabile mesodermal agent, and the 
epidermal agents, which are present in the inductor, have consequently been 
enabled to exert their own effect.

6. The chemical nature of the mesoderm-inducing agent is discussed; and also 
the similarity of the tissue-specific agents which presumably regulate regenera-
tion in adult vertebrates and the embryonic inducing agent.

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EXPLANATION OF PLATES

PLATE 1

FIG. A. Section through an embryo with a lens induced by heat-treated bone-marrow.

FIG. B. Section through an embryo with a balancer and a lens, both induced by heat-treated bone-marrow.

FIG. C. Section through an embryo in which a lens with two fibre centres and a balancer have been induced by heat-treated bone-marrow.

FIG. D. Section through an embryo with a balancer induced by heat-treated bone-marrow.

FIG. E. The heat-treated bone-marrow has induced normal epidermis and a lentoid in an epidermal explant in the neighbourhood of the inductor; elsewhere the epidermis has remained atypical.

PLATE 2

FIG. A. The alcohol-treated bone-marrow has induced in an epidermal explant four forelimb rudiments and a tail-like formation.

FIG. B. A section through the same explant where the inductor has induced notochord, myotomes, pronephric tubules, a fin containing ectomesenchyme and a proctodaeum with anal opening.

FIG. C. Another section through the same explant with notochord, myotomes, pronephros, and a forelimb rudiment.

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