sich bildende weisse Niederschlag wird abzentrifugiert und in wenig Wasser aufgenommen. Die Lösung enthält rund 90% der eingesetzten Kallidinmenge. Diese empfindliche Fällungsreaktion dürfte auch für die Isolierung von Kallidin von praktischer Bedeutung sein.

JAQUES et al.[7] haben kürzlich festgestellt, dass auch Hypertensin durch Heparin gebunden wird. Da ausser Histamin, Hypertensin und Kallidin anscheinend auch Thrombin[8] mit Heparin einen Komplex bildet, nehmen wir mit JAQUES et al. an, dass der Bildung von Heparin-Komplexen eine allgemeinere Bedeutung zukommt.

Summary. Kallidin gives, with heparin, a non-dialysable complex, which can be precipitated with alcohol. In this binding, kallidin is pharmacologically active, but may be split off by the substance 48/80.

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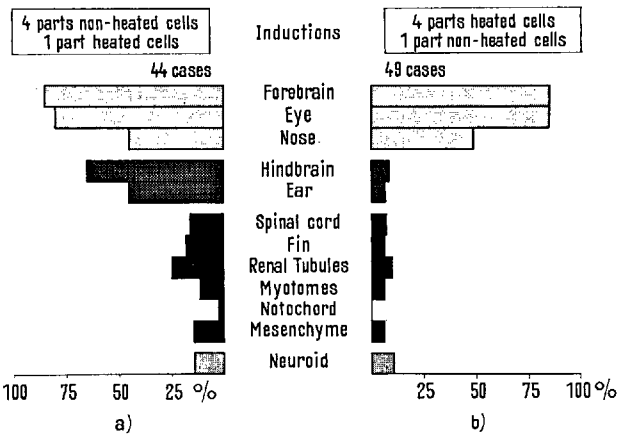
Quantitative Evidence for the Two-Gradient Hypothesis in the Primary Induction

The idea of a two-gradient system acting in the primary induction, presented by LEHMANN[1] and YAMADA[2], had earlier led us to experiments in which two heterogeneous tissues of qualitatively different inductive action were implanted simultaneously in order to test their combined effect[3]. A new type of induction was observed, explained as being a combined action of two principles, one a mesodermalizing and the other a neuralizing inductive system. A very similar theory was simultaneously presented by YAMADA and TAKATA[4].

These ideas were based on two types of experiment: The combination of two different inductors, or a separation of the two different inductive effects by chemical treatment of one inductor tissue, originally 'combined'. None of these experiments, however, could give us quantitative information on the hypothetical principles operating in the primary induction. In such a quantitative operation, our present methods with their marked limitations seem to offer only two possible ways of approach; either the size of the implant is varied, or different concentrations of the active factors incorporated in some non-inducing material are used. For a number of reasons, both of these methods have to be considered inadequate, and also the techniques give rise to a variety of technical errors. Another set-up was accordingly planned so as to get at least some relative data on the amount of the different inducing principles, and the significance of their ratio to the induction process.

Method. HeLa-cells cultured in a medium containing human serum were used as inductors[5]. After mechanical removal of the cells from the walls of the culture flasks, they were treated as follows:

From 10 to 11 million cells were collected from 3 culture flasks, washed several times with saline solution, and finally dispersed in 10 cm³ of saline. Of this homogeneous cell suspension, 5 cm³ was heated on a waterbath for 30 min at 65°—a treatment which is known to inactivate the mesodermalizing capacity of cells and tissues[5,6]. Meanwhile, the other half of the suspension was kept in an incubator at 37°C. Following this, the two suspensions were combined in two test tubes in the following ratios: A) 1 cm³ heated cells + 4 cm³ non-heated cells; B) 4 cm³ heated cells + 1 cm³ non-heated cells.



Percentage of the induced structures in the experimental series.

During the whole of this procedure, every care was taken to keep the cell suspensions dispersed homogeneously, and the tubes were repeatedly shaken between the different stages of treatment. The two combined suspensions (A, B) were then simultaneously centrifuged at 5000 rpm, and the compact sediment covered with cold 70% alcohol. After 4 to 6 h of alcohol treatment, the cells were washed and used in explantation experiments according to 'sandwich' method[7]. The presumptive epidermis of young Triturus vulgaris-gastrulae was used, and the explants were cultivated for 10 days in Holtfreter-solution. The differentiations were analyzed microscopically from serial sections.

Results. The results are demonstrated in the Figure. Only one comment has to be made as regards the Figure. As was the case in our earlier works, only the presence or absence of the different induced structures was recorded, and thus the analysis gives no information on the amount or size of the secondary formations. For instance, the large masses of striated muscle seen in some cases in series A were recorded as being equal to the explants in series B, where often only a few muscle fibers were found. Thus, in this respect the title of our report is misleading, and the analysis of the inductions is by no means quantitative.

Discussion. In comparison with our earlier findings obtained with HeLa-cells in implantation experiments[5], the low frequency of mesodermal structures was conspicuous in both of the present series. This is partly correlated with the generally known fact that mesodermalization is always weaker in explantation experiments than in those of implantation[3,8]. Furthermore, we know from our earlier and partly unpublished experiments that a maximal mesodermalizing effect is noted in HeLa-cells, only if their culture medium is changed shortly before the experiment.

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In the present experiments, the cells were grown for three days in the same culture medium. Accordingly, the results can hardly be compared with our earlier observations[5], and a comparison will be made only between the two present series, A and B.

By reason of the small number of mesodermal differentiations in both series, the difference is not very striking, but the greater number of all spinocaudal structures in series A can be noted. The most striking difference is in the number (and size) of the deuterencephalic structures. According to our two-gradient hypothesis[3], the deuterencephalic structures are developed as a result of an initial stimulus of two inductive principles, the neuralizing and the mesodermalizing principle. Furthermore, we know that the mesodermalizing factor(s) is inactivated by heat treatment, and is thus not active in the heated HeLa-cells[5,6]. The hindbrain structures seen in some cases of series B must consequently be a result of a neural induction together with the mesodermalizing effect of the small amount of non-heated HeLa-cells in the implant. When the relative amount of the mesodermalizing factor(s) is increased by changing the ratio of heated and non-heated cells in the inductor, there appears a definite increase in the number (and size) of the deuterencephalic structures (series A). We are therefore inclined to conclude that the regional type of induction is here not dependent only on the presence or absence of certain inductive principles, but that it is also a reflection of the relative amounts of these active factors.

Our modification of the two-gradient hypothesis has recently been criticized and considered as an 'oversimplification'[9]. We agree in that it is a simplified model, but we should like to stress that it is only a working hypothesis, and not intended to form an explanation of the most complicated process of primary induction. So far, it seems to explain the earlier findings on relations between the inducing principles and the induced structures.

The quantitative experiments will be continued employing different ratios of heated and non-heated cells, and in the presentation of these results the problem will be discussed in more detail.

Zusammenfassung. Die Einwirkung quantitativer Unterschiede induzierender Agenzien wurde untersucht. HeLa-Zellen, mit und ohne Wärmebehandlung, wurden in zwei verschiedenen Proportionen (4:1 bzw. 1:4) gemischt und als Induktor in Epidermisexplantaten des Molchkeimes benutzt. Das Resultat (Fig.) zeigt, dass der Induktor, der mehr Zellen ohne Wärmebehandlung enthält, mehr mesodermale, aber auch mehr deuterencephale Gebilde induziert. Das Resultat wird mit der Zwei-Gradienten-Hypothese der induzierenden Agenzien erklärt.

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Department of physiological Zoology, University of Helsinki (Finland), November 8, 1960.

An Experimentally Produced Change in the Sequence of Neuralizing and Mesodermalizing Inductive Actions

According to Nieuwkoop et al.[1], the regionality of the central nervous system is caused by two successive stimuli. The first of these leads to the non-specific 'activation' of the competent ectoderm, which reacts, differentiating to the archencephalon and its derivatives only. The second stimulus then causes the 'transformation' of the 'activated' ectoderm, resulting in a modification of the archencephalic differentiation tendencies in structures typical of more caudal regions of the nervous system. Nieuwkoop states that the 'transformation', i.e. the differentiation of more caudal formations of the central nervous system, occurs only if the ectoderm is previously 'activated'.

In our opinion[2,3], two different principles are active in the primary induction, neuralizing (N), and mesodermalizing (M). The N principle, if it acts alone, causes the differentiation of the archencephalon and its derivatives. The M principle correspondingly brings about the mesodermal differentiations, if acting alone. But, in the normogenesis, these two principles form two partly overlapping gradients. This system would direct the regionality of all the structures induced.

I have shown earlier[3], in experiments with heterogenous inductors, that under experimental conditions the mesodermal differentiations can be caused with no previous neuralizing action ('activation' of Nieuwkoop). In order to test the independent characteristics of these two principles assumed by us, I arranged experiments in which an attempt was made to change the normal sequence of the actions of these two principles.

Material and methods. The 'sandwich' method was used. The host epidermis was from young gastrulae of the common newt (*Triturus vulgaris*). The inductor materials were marrow from the thigh-bone of the guinea-pig, and HeLA cells[4] cultured *in vitro*. The bone marrow tissues was fixed in 70% alcohol for 24–72 h, and washed in sterile saline for 1–2 h before use in experiments. The HeLa cells were cultured in Roux flasks in a growth medium consisting of 30% pooled fresh human serum, and 70% Hanks' solution[5]. The cells adhering to the walls of the Roux flasks were mechanically detached without trypsinization, centrifuged, washed three times in buffered saline, and heated in glass tubes for 30 min at 70°C in a water bath. Subsequently, the cells were fixed in alcohol and washed in sterile saline in the same way as the bone marrow mentioned above. The Holtfreter saline for culturing the explants was buffered with phosphates[6]. The explants were cultured for 10 days. In experimental series B and C (Fig.) the explants were opened, and the inductor was removed after cultivation for 3 h. In series C, the bone marrow tissue was replaced with HeLa cells. The explants were fixed in Bouin's fluid, first stained in bulk with borax-carmine, and the serial sections counter-stained with picro-blue-black.

Results. A detailed analysis of the structures induced and the arrangements for the experiments is given in the Figure.

Series A and D are controls which show the inductive actions of both of the inductors used. The bone marrow induced almost mesodermal structures. The spinal cord was the only neural formation induced. It was always short and thin, and on the tip of the induced tail only. The heated HeLa cells were strictly pure inductors of archencephalic structures.

If the bone marrow was removed after 3 h (B), the explant differentiated to all the types of differentiations as in series A, but less frequently. The lower frequency of the

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