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.

S. Toivonen[10], L. Saxén, and T. Vainio

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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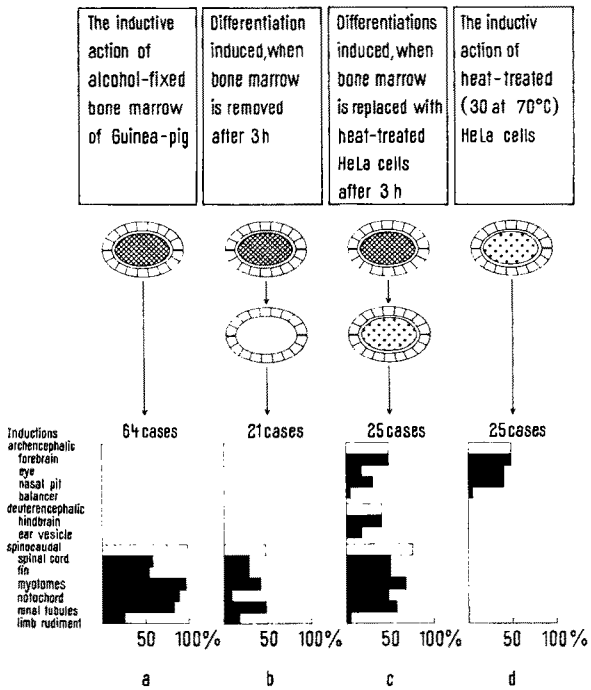

The arrangement of the experiment and the analysis of the structures induced in the different series.

differentiations compared with the control series (A) might in part have depended on the fact that the inductor tissue was somewhat infiltrated with living cells of the host, and, when the inductor was removed, some of the determined cells were removed with it.

Series B is also a control for the 3 h action of the bone marrow in series c, where it was replaced with the heated HeLa cells. In this series, deuterencephalic differentiations were also noted, although they were lacking completely in the other series. One has to conclude that they were induced by a simultaneous action of both of the inductors, which were separately unable to induce them, a fact which we have noted earlier[2]. Another fact one might consider in this connection is that the differentiations of the mesodermal structure in series C were more frequent than in the corresponding control (B). The fact that the mesodermal differentiation is stimulated by the presence of neural structures, has been observed earlier[7,8].

Discussion. The above results corroborate our earlier opinion that the mesodermalizing and the neuralizing inductive actions are caused by different principles, because their normal sequence can be experimentally reversed, that is, to use the terminology of NIEUWKOOP, there may occur a mesodermalizing 'activation' and neuralizing 'transformation'.

As a matter of fact, it has to be admitted that the difference between the views of NIEUWKOOP et al.[1] on the one hand, and of myself and my co-worker[2] on the other, is largely a terminological one, as I have pointed out earlier[3]. NIEUWKOOP's non-specific 'activation' process corresponds to the action of our N principle, and his 'transformation' process appears to correspond to the action of our M principle. But, as these two actions can be separated one from the other, as the specific actions of the inductors used in the experiments above indicate, and as the sequence of these two actions can be reversed, in my opinion the terminology of NIEUWKOOP is no longer quite adequate.

For the present, it seems that our two-gradient-hypothesis[2], which is similar to that presented by LEHMANN[9] and YAMADA[10], provides a satisfactory explanation of the regionality caused by the action system in the primary induction process.

Zusammenfassung. Die normale Reihenfolge der neuralisierenden und mesodermalisierenden Aktionen wurde experimentell umgekehrt. Im Explantatversuch diente die präsumptive Epidermis des Molchkeimes als Reaktionsmaterial. Als neuralisierender Induktor wurden HeLa-Zellen nach Wärme- und Alkoholbehandlung, und als mesodermalisierender das Knochenmarkgewebe des Meerschweinchens nach Alkoholbehandlung benutzt. Die Anordnung der Versuche sowie die Analyse der induzierten Gebilde sind in der Figur dargestellt. Das Resultat wird mit der Zwei-Gradienten-Hypothese der induzierenden Agenzien erklärt.

S. TOIVONEN[11]

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First Results of Tissue Culture in *Drosophila*

It is well known that it is possible, after 4–5 day culture, to achieve *in vitro* differentiation of imaginal discs of the eyes and wings of *Drosophila melanogaster* (DEMAL[1], GOTTSCHEWSKI[2], GRACE[3], HORIKAWA[4–7], HORIKAWA and SUGAHARA[8], KURODA[9–11], KURODA and YAGAMUCHI[12], KURODA and TAMURA[13]). HORIKAWA and SUGAHARA[8] kept in a culture for 72 h also salivary glands, testicles, fat bodies and cephalic complexes, while HORIKAWA and KURODA[14] succeeded in obtaining an active multiplication of the haemolymph cells for two weeks.

Undifferentiated tissue of the larval central nervous system and cells obtained from the lymph gland seemed to us to be the most suitable material for further attempts while experimenting with various types of culture medium. The basic elements that we used in the composition of the culture media were: plasma of heparinized cock's blood (P); extract of chick embryo after 8–9 days' incubation (EE); synthetic medium for insects according to Grace (PAUL[15]) without cholesterol (SM); extract of *D. melanogaster* larvae of the wild Varese or Aspra 52 strains (ELV, ELA). Antibiotics were added to the cultures.

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