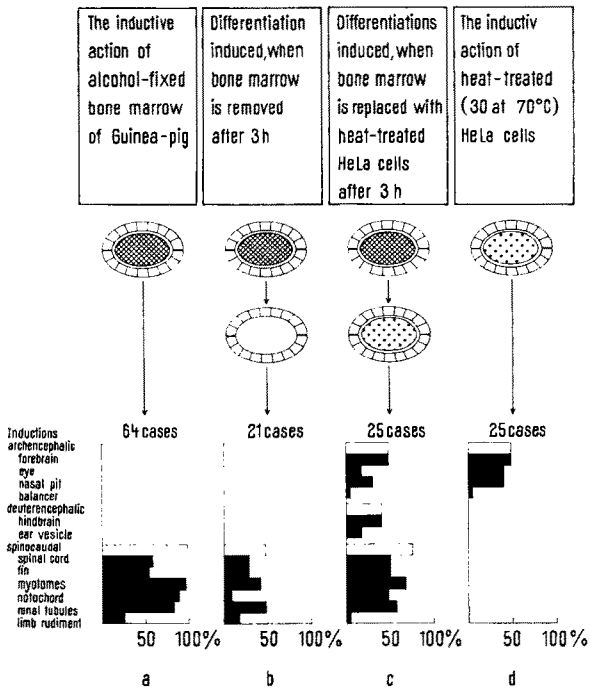

The arrangement of the experiment and the analysis of the structures induced in the different series.

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.

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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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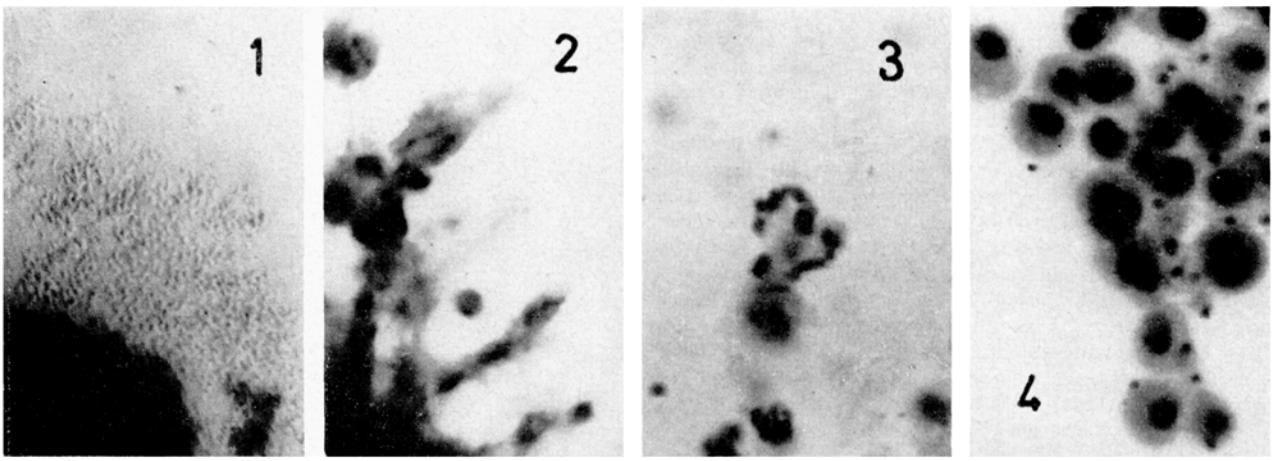

Fig. 1. Cell migration from a Varese ganglion (9th day) in phase contrast (× 140). – Fig. 2. Cell migration from a Varese ganglion (19th day) stained with Delafield's haematoxylin (× 1400). – Fig. 3. Mitosis in Aspra ganglion (17th day) stained with orcein acetic (× 1650). – Fig. 4. Cells from a lymph gland of S. Maria strain (10th day) stained with May Grümwald-Giemsa (× 1200).

In the experiments with embryo extract, Waddington's physiological solution (pH 7.3) was used, while in the experiments with synthetic medium we preferred to use the same solution of salts that makes up part of the medium itself (pH 6.6). The cultures were prepared by the hanging drop method, preserved in a thermostat at 25°C ± 0.5 and transplanted every 7 days.

The best results were obtained with the cephalic ganglia (2 cerebral hemispheres and ventral ganglion), and are summarized in the Table.

The condition of the cultures was judged on the basis of observation both by phase contrast microscope and of material fixed in Carnoy and stained with Delafield's haematoxylin. Mitoses were observed also on crushed material stained with acetic orcein.

It is evident that we managed to obtain survivals of 4–5 weeks, although the optimum period is limited to between 14 and 21 days. In fact it is during the second week of culture that the spreading of cells outside the ganglion is noted (Fig. 1 and 2), at the same time as the appearance of some mitotic figures, especially in Aspra larvae ganglia (Fig. 3). The latter tend to form more globous and thicker expansions in comparison with Varese or S. Maria larvae ganglia.

With regard to the results obtained with a culture medium composition that can be said complete (P + EE + + EL or P + SM + EL), survival is reduced noticeably in the absence of larvae extract, and made impossible with only embryo extract or only synthetic medium. The lack of plasma, on the other hand, brings about a precocious lysis of the ganglion membrane with consequent liberation of the cells in the culture medium. This phenomenon is particularly evident in the synthetic medium. In this case, we succeeded in obtaining the survival of isolated cells for 39 days: however no mitosis was seen. The inability of isolated cells to multiply is justified by a similar fact already noted in mammals (Sato, Fisher and Puck[16]). In fact it was observed how the same culture medium which was sufficient for organs or tissue fragments had to be further enriched with certain substances in order to cultivate various types of isolated cells.

A similar explanation may be given of the results obtained by cultivating lymph glands of the S. Maria strain. This gland is particularly fragile and allows the isolated cells to escape easily. The latter survive in the culture media experimented by us for 10–13 days without mitoses being observed (Fig. 4). Sometimes they take on an ameboid aspect.

Stocks	Culture medium	Age of cultures in days 7	14	21	28	35
I 1. Varese – S. Maria	P + EE + ELV	+	++	++	+–	
2. Aspra	P + EE + ELV	+	++			
3. Aspra	P + EE + ELA	+	++			
II 4. Varese – S. Maria	P + SM + ELV	+	++	++	+–	
5. Aspra	P + SM + ELV	+	++	++	++	+–
6. Aspra	P + SM + ELA	+	++	++	+–	
III 7. Varese – S. Maria	P + SM	++	++	+–		
8. Aspra	P + SM	++	++	+–		
IV 9. Varese – S. Maria	EE + ELV	+	++			
10. Aspra	SM + ELA	++	+++	+++	++–	++–
11. Varese – S. Maria	P + ELV	––				

\+ = compact ganglion – living cells.
++ = ganglion with expansions – living cells – pyknotic nuclei and mitoses.
+– = ganglion with expansions – cultures only partially living
–– = dead ganglion
+++ = dissolved ganglion – living free cells
++– = free cells – partially living culture.
––– = dead free cells.

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The lymph glands of Varese strain, with compact structures, survive for an even shorter length of time; thereafter part of the cells undergo processes of turnig black, which are similar to those that transform haemolymph cells into pseudotumours.

In this respect, it has been seen that the large haemolymph cells (CASTIGLIONI[17]), which have already aggregated to form tumours (Aspra 52 strain) and are kept in a culture for 15 days, are subject to progressive melanization. Also other workers, who have tried to cultivate melanotic tumours, have observed a progressive expansion of melanization in cells near the tumour (KURODA and TAMURA[18–25]) or in cells which have migrated into other tissues from the tumour itself (FRIEDMAN and BURTON[26], FRIEDMAN et al.[27]) without this proving that there was any cellular multiplication. In our experiments, in one culture alone was a bridge formed of living cells (some probably in mitosis) between two fragments of tumours.

While admitting the preliminary nature of the present research, we believe we can point, as being of some interest, to the results obtained by us up to date, especially those on ganglia, both for the relatively long survival period and for the documented growth through mitotic divisions. This preliminary research will allow us next to try to obtain clone cultures on culture media kept under the strictest control.

Riassunto. Gli autori riescono ad ottenere la sopravvivenza in cultura di gangli cefalici larvali di *Drosophila melanogaster* per oltre un mese, con mitosi durante la 2ª e 3ª settimana, mentre le ghiandole della linfa si mantengono vive solo per 10–13 giorni.

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Stretching Activity in Dogs Intracisternally Injected with a Synthetic Melanocyte-Stimulating Hexapeptide

In dogs, intracisternal injections of highly purified ACTH preparations induce typical, prolonged, and repeated stretching crises[1,2]. Similar effects are caused by purified MSH preparations[3] and by solutions of ACTH made with NaOH $N/10$ treated at 100°C for 20 min[2]. These findings suggest that the chemical groups eliciting the stretching responses are related with those stimulating melanocytes. This correlation is supported by results obtained with dogs given intracisternally the acetate salt of the synthetic hexapeptide H Glu(NH_2)-His-Phe-Arg-Try-Gly-OH with MSH-like activity. 750 γ/kg or less of this peptide (given intracisternally) evoke stretching responses similar to those following injections of MSH or ACTH. 1.5 mg/kg of the peptide causes lasting depression and scialorrhea but not a stretching crisis.

The melanocyte stimulating effect of the peptide is 2×10^5 U/g[4]; that of a purified MSH preparation (732179 A by Armour Laboratories, Chicago, Ill.) 5×10^3 U/g. The threshold dose for the stretching response was respectively 500 and 5 γ/kg: indeed these two different pharmacological actions are strictly related. The hexapeptide, but not MSH, shows a paradoxycal behaviour and a pharmacological response evoked by the polypeptide may change qualitatively with respect to the dose given intracisternally.

The Figure illustrates a typical stretching crisis in a dog injected intracisternally with the hexapeptide.

A typical stretching crisis in a dog injected intracisternally with the melanocyte-stimulating hexapeptide

Riassunto. La iniezione endocisternale nel cane di dosi fino a 0.75 mg/kg del sale acetico dell'esapeptide H.Glu(NH_2)-Ist-Fen-Arg-Tript-Gli.OH, svolgente un'attività melanoforo-stimolante della intensità di 2×10^5 U/g, induce delle tipiche crisi di stiramento, del tutto simili a quelle che si ottengono iniettando per la stessa via dell'ACTH o dell'MSH. Dosi di 1.5 mg/kg invece non inducono più crisi di stiramento ma deprimono notevolmente gli animali e determinano una intensa scialorrea. Sembra di poter ammettere che la struttura chimica responsabile dell'effetto sui melanociti sia anche responsabile dell'induzione delle crisi di stiramento.

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The Effect of Parabiosis and Fluid Restriction on the Development of Azo Dye Induced Rat Liver Tumors[1]

CAMPBELL and STONE[2] have shown that slices of liver tumor synthesize 1/3 to 1/2 the amount of serum albumin produced by slices of normal liver. GLINOS[3] has provided evidence that depletion of the plasma protein level by plasmapheresis can accelerate liver regeneration in the rat and that fluid restriction retards liver regeneration by increasing the relative concentration of plasma proteins.

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