	Injected larvae	Hatched flies	Without tumor	With tumor	% of tumor flies
Physiological solution in *Varese* (0%)	252	112 (44.44%)	111	1	0.89
Haemolymph of *tu-w* (40%) in *Varese* (0%)	164	82 (50%)	69	13	15.85
Haemolymph of *tu-w* (80%) in *Varese* (0%)	147	83 (56.46%)	51	32	38.55
Haemolymph of *Varese* (0%) in *tu-Oregon* (1%)	311	137 (44.05%)	134	3	2.18
Haemolymph of *simulans* (98%) in *Varese* (0%)	284	9 (3.16%)	4	5	55.55
Haemolymph of *simulans* (0%) in *Varese* (0%)	122	5 (4.09%)	5	0	0

Varese (0%) = normal *melanogaster* stock without tumors. – *tu-w* (40%) = white-eyed flies carrying tumors at an incidence of 40%. *tu-w* (80%) = white-eyed flies carrying tumors at 80%. – *tu-Oregon* (1%) = Oregon carrying tumors at 1%. – *simulans* (98%) = *D. simulans* stock having a tumor incidence of 98%. – *simulans* (0%) = normal *simulans* stock without tumors.

(3) injections of haemolymph from a tumorless stock into a low incidence stock;

(4) injections between different species (*melanogaster-simulans*), by using normal *melanogaster* larvae as hosts and *simulans* ones, both with and without tumors, as donors.

First it follows that, owing to injections of haemolymph from tumor stocks into normal larvae (Varese), the hatching flies can have tumors. We might now suppose that these melanotic masses are due merely to the traumatic effect of the operation: that can be ruled out by the control experiment, i.e. injecting physiological solution. Negative results in this series of experiments seem to annul the possibility of any mechanical consequence of the injection. Regarding this point, it is also a very significant fact that the number of induced pseudotumors is roughly proportional, if not equal, to the incidence of the donor stock.

Nevertheless, the hypothesis of a virus infection, carried by the blood, might still be open. In that case, the occurence of tumors would not be linked to hereditary factors, while the genetical mechanisms determinating the transmission of this character appear to be only genic (BARIGOZZI[1]).

From the facts mentioned above, a fundamental role in the forming of pseudotumors may be attributed to the haemolymph.

It now remains to be seen how the haemolymph acts.

We do not know whether the hypothesis of a kind of reaction between the blood of different stocks can be made, in order to explain the induction of pseudotumors in *Drosophila*. But, at any rate, the results of the injections of haemolymph from normal stock into a low incidence stock are against such a hypothesis. In fact, the normal haemolymph acts like physiological solution, and is even unable to increase significantly the incidence of tumors in a stock already genotypically determined for their production.

Besides this, it must be remarked that in the experiments of reciprocal injections between different stocks of the same species (melanogaster), no matter whether with or without tumors, the frequence of survivors after operation is always similar to that of the control experiment with physiological solution (about 50%). Therefore death is not caused by the properties of the injected fluid.

The results differ, when the injections take place between different species: there is a certain incompatibility between *simulans* and *melanogaster* haemolymph, and the percentage of survivors is reduced to 3–4%. The production of pseudotumors sometimes also occurs, nevertheless, between these species. Also in the latter case, a donor stock with high incidence of tumors produces tumors in the host.

Nevertheless, it still remains open whether the injected cells keep their particular property of aggregating and melanizing, even in a different environment, or whether it is the injected fluid as a whole which acts on the host's cells. At present this question cannot be answered. We can only say that, after a first series of orientative countings, a significant difference in the number of blood cells has been found between a tumor and a tumorless stock; the same is probably true also between tumor and tumorless larvae of the same stock.

This might justify the hypothesis of an abnormal multiplication of blood cells before the formation of tumors. Thus the injected haemolymph from a tumor larva would be more active in producing melanotic masses, being rich in cells. A high number of blood cells might be a necessary but not a sufficient condition for the formation of pseudotumors, because, for producing a pseudotumor, cells must also be able to melanize.

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Institute of Genetics, University of Milan, Italy, June 10, 1954.

Riassunto

Gli autori illustrano una prima serie di esperimenti di iniezioni reciproche di emolinfa tra ceppi con e senza pseudotumori in *Drosophila melanogaster* e *simulans*.

Soluzione fisiologica ed emolinfa da ceppo sano non determinano formazione di pseudotumori. Emolinfa da ceppo portatore di pseudotumori induce gli stessi in ceppo normale con una frequenza pressocchè proporzionale all'incidenza nel ceppo donatore: questo avviene anche con iniezioni intraspecifiche.

Inoltre sembra che la presenza di pseudotumore sia accompagnata da un maggior numero di elementi cellulari presenti nell'emolinfa della larva.

Cultivation of Embryonic Organ Rudiments on a Medium Derived Entirely from Adult Tissues

Many embryonic organ rudiments, when explanted and cultivated *in vitro* under suitable conditions, continue to develop and sometimes attain a remarkable

[1] C. BARIGOZZI, Proc. IX Int. Congr. Genetics (in press).

degree of histological and anatomical differentiation[1]. The type of tissue culture promoting "organized growth" therefore appears most suitable for studying the inherent developmental potencies or the capacity for self-differentiation of embryonic organ rudiments under conditions of isolation from the embryo. However, by following the usual procedure of growing the explants in media containing embryo extract, the humoral isolation of the rudiments is only partially achieved. The question is therefore raised whether the characteristic differentiation of explants under such conditions depends on the presence of embryonic materials in the culture medium, or whether they are capable of a similar development *in vitro* in absence of any embryonic tissue derivatives.

To test these alternatives, embryonic organ rudiments were grown on a culture medium in which an extract from adult fowl heart tissue was substitued for the embryo extract. This attempt was based on previous information that the addition of extracts of adult tissues to the culture medium resulted in improvement of growth, migration[2] and the maintenance of structure of adult[3] and of juvenile[4] tissue explants.

Material and Methods. Limb-buds of 4-day chick embryos and pituitary rudiments of 6-day embryos were cultivated by the watch glass technique[5] on a culture medium, consisting of 6 parts of fowl plasma and 3 parts of adult fowl heart tissue extract. Both components were mixed in the watch glass and transferred to an incubator at 38°C, where clotting took place usually in 10 to 15 min.

The heart extract was prepared by the method described by MARGOLIASH[6]. The minced fresh heart muscle tissue was dehydrated with several changes of acetone, then dried and ground to a powder. This could be stored in the refrigerator for several months without apparent loss of activity. To prepare the extract, the desiccated heart tissue was extracted with 22 volumes of TYRODE's solution for 24 h at 4°C; the resulting solution was then sterilized by filtration through a SEITZ filter.

The detailed procedure of dissection of the rudiments and of their cultivation *in vitro* were described elsewhere[7].

Results. The mesoblast of the 4-day chick limb-bud consisted, at the time of explantation, of structurally non-differentiated mesenchyme. During 5 days of cultivation on the plasma-adult heart extract medium the rudiments developed into the proximal skeletal elements of the limb (Fig. 1). The differentiated explants consisted of the cartilaginous structures of the knee-joint region, the cartilage presenting a normal histological appearance. The development and histogenesis of these cultures followed essentially the same course as that described for limb rudiments cultivated on the standard plasma-embryo extract medium[1].

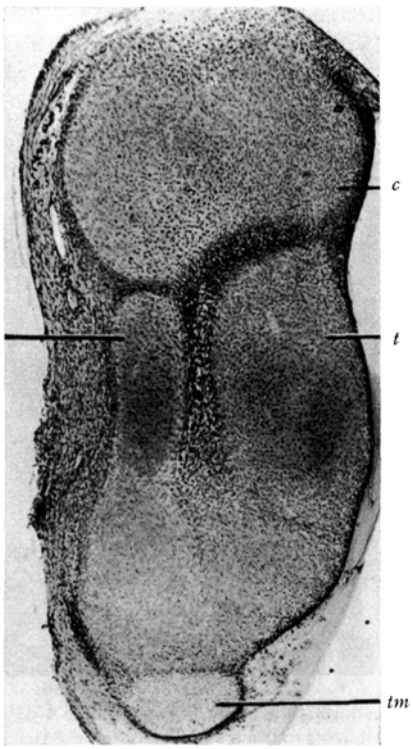

Fig. 1.–Section through a mesoblast of a 4-day leg-bud after 5 days' cultivation on plasma-adult heart extract, showing differentiation of skeletal elements: *c*, femoral condyli; *t*, tibia; *f*, fibula; *tm*, tarsus-metatarsus. DELAFIELD's haematoxylin, eosin. × 38.

The anterior lobe rudiment of the 6-day chick pituitary consisted, at the time of explantation, of an epithelial vesicle. During 8 days of cultivation on the plasma-adult heart extract medium it developed into typical anterior lobe tissue. The cellular cords, surrounded by connective tissue, contained numerous characteristic basophil and acidophil cells (Fig. 2). The histological and cytological differentiation of these explants was essentially similar to that of rudiments cultivated on plasma-embryo extract medium; moreover, explants grown on the heart-extract containing medium showed a more pronounced differentiation of the secretory cells.

All the cultures on the heart extract-plasma medium showed good fibroblastic outgrowth and the usual liquefaction of the clot. Concurrently with the progressive differentiation of the explanted rudiments, there was a marked increase in the volume of the tissue indicating assimilation and utilization of the culture medium by the explants.

Comments. The above experiments demonstrated that a culture medium derived solely from the tissues of an adult supported the typical development *in vitro* of the two types of embryonic organ rudiments studied. This implies that the endogenous capacity of the rudiments for self-differentiation can be realized *in vitro* in the absence of embryonic tissues derivatives from the culture medium. It should, therefore, be assumed that the metabolic requirements of the cells are satisfied by the

[1] H. B. FELL, *Histogenesis in Tissue Culture*, in *Cytology and Cell Physiology* (G. H. Bourne, Clarendon Press, Oxford, 1951); J. R. Micr. Soc. *60*, 195 (1940).

[2] R. S. HOFFMANN, E. TENNENBAUM, and L. DOLJANSKI, Growth *4*, 207 (1940). – E. MARGOLIASH, Science *105*, 369 (1947).

[3] L. DOLJANSKI, R. S. HOFFMAN, and E. TENNENBAUM, Nature *150*, 23 (1942). – O. ULLOA-GREGORI, T. G. BLOCKER, JR., W. W. NOWINSKI, and C. M. POMERAT, Texas Reports on Biol. Med. *8*, 400 (1950).

[4] P. J. GAILLARD, *Organ Culture Technique Utilizing Embryological Watch Glasses*, in *Methods in Medical Research 4* (M. B. VISSCHER, ed., Year Book Publishing Co., Chicago, 1951).

[5] H. B. FELL and R. ROBISON, Biochem. J. *23*, 767 (1929).

[6] E. MARGOLIASH, Science *105*, 369 (1947).

[7] A. MOSCONA and H. MOSCONA, J. Anat. *86*, 287 (1952). – H. MOSCONA and A. MOSCONA, J. Anat. *86*, 278 (1952).

[1] H. B. FELL and R. ROBISON, Biochem. J. *23*, 767 (1929). – A. MOSCONA and H. MOSCONA, J. Anat. *86*, 287 (1952).

components of the adult tissue derivatives present in the medium. If any specific "embryonic substances" are necessary for the orderly development of embryonic

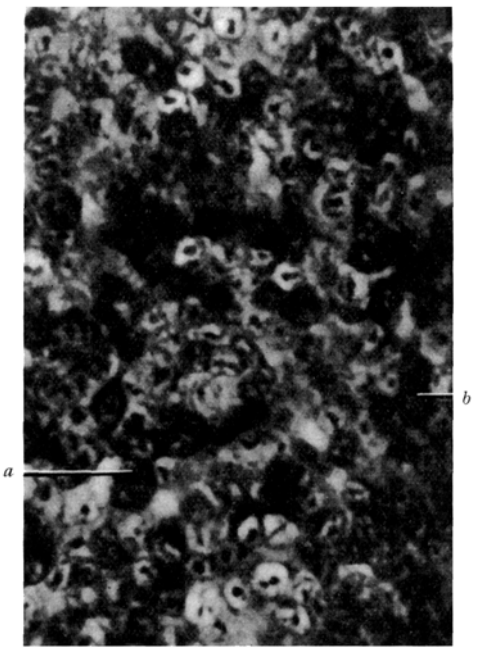

Fig. 2.–Section through a 6-day pituitary after 8 days' cultivation on plasma-adult heart extract, showing differentiation of lobules and chromophilic cells: *a*, acidophil, and *b*, basophil cells. Azan. × 1140.

tissues *in vitro*[1] they must have been, in this case, either present in the explants or produced by them under the conditions of cultivation on the "adult" medium. It should be stressed, however, that the developmental fate of the tissues studied here had already been determined at the time of explantation and that different results may obtain with explants at earlier stages of development. This aspect of the problem is at present under further study.

We wish to take this opportunity to express our thanks to Dr. HONOR B. FELL, F.R.S., Director of the Strangeways Research Laboratory, Cambridge, for introducing us to the tissue culture technique of organ rudiments.

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Department of Zoology, The Hebrew University, Jerusalem, April 14, 1954.

Zusammenfassung

Extremitätenknospen von viertägigen und Hypophysenanlagen von sechstägigen Hühnerembryonen wurden explantiert und *in vitro* kultiviert. Das Kulturmedium bestand aus Blutplasma und einem Tyrodeextrakt des Herzmuskels vom adulten Huhn. Die untersuchten Embryonalanlagen zeigten normale Entwicklung und Gewebsdifferenzierung, obwohl das Milieu gar keine embryonalen Zusätze enthielt.

[1] A. CARREL and A. H. EBELING, J. Exp. Med. *38*, 499 (1923).

Weiteres zum Lichtsinn augenloser Muscheln

(Versuche mit *Anodonta cygnea* und *Pseudanodonta complanata*)

Eigene Experimente[1] haben es wahrscheinlich gemacht, dass auch augenlosen Muscheln neben der Wahrnehmung von Hell-Dunkel und Beschattung ein «Bewegungssehen» zukommt. Bei *Cardium oblongum* und *Mactra stultorum* waren in eigenen und in den histologischen Studien anderer Autoren[2] keine Sehorgane aufgefunden worden, dennoch zeigten diese Muscheln ein «Bewegungssehen».

Die beiden Süsswassermuscheln *Anodonta cygnea* und *Pseudanodonta complanata*, bei denen mit Sicherheit keinerlei augenähnlichen Organe vorkommen, zeigten in verschiedenen Versuchsanordnungen weder eine positive noch eine negative Phototaxis. – Auf Zunahme der Lichtintensität reagierten beide Muscheln nicht. Im Zweilichterversuch zeigten sie aber Reaktionen auf Verdunkelung (Tab. I). Die Tiere reagierten selbstverständlich auch dann auf Verdunklung, wenn diese mit einer Beschattung verbunden war. Diese wurde mit geeichten Jenaer Grauscheiben erzeugt (Absorptionswerte von 10 bis 90%). Die Scheiben wurden möglichst schnell (siehe unten) vor den konzentrierten, auf die Siphonalregion der geprüften Muscheln gerichteten Lichtstrahl geklappt. Die Reaktion der Muscheln bestand im Zusammenlegen der reusenartigen Tentakeln der Siphonalregion. Ein Vergleich mit anderen von uns untersuchten Muscheln[3] charakterisiert die beiden Anodontiden als «gutsehende» Muscheln (in bezug auf Verdunkelungs- und Beschattungsempfindlichkeit).

Bei *Anodonta cygnea* wurden die ersten Reaktionen bei einer Grauscheibenverdunkelung (und Beschattung) von 9,1% beobachtet. Sie traten auf, wenn die Grauscheibe schnell, das heisst im Bruchteil einer Sekunde, vorgeklappt wurde und somit der Faktor «Bewegung» (im Rahmen des Üblichmessbaren) nicht in Erscheinung treten konnte. Bei einer Grauscheibenverdunkelung von 8,3% aber konnte so keine Reaktion bei dieser Muschelart mehr beobachtet werden. Wurde diese Grauscheibe aber langsam und mit gleichbleibender Geschwindigkeit (Transport durch ein regulierbares Kymographion) durch den Lichtstrahl geführt, so dass die Verdunkelung (und Beschattung) sich langsam über die siphonale Region des Tieres bewegte, so traten nunmehr Reaktionen auf, praktisch ohne Latenz. Dies weist auf einen Einfluss der Bewegung hin. Bewegte, kompakte Schattenstreifen lösten stets eine besonders heftige Tentakelreaktion aus, bewegte, sehr schwach absorbierende Grauscheiben (10%) dann, wenn ihre Bewegung eine gewisse Schnelligkeit nicht überstieg (Tab. II, III). Auch wenn viel stärker verdunkelnde (und beschattende) Grauscheiben (20–50%) mit relativ hoher Geschwindigkeit durch den Lichtstrahl geführt wurden, blieben Reaktionen aus. Die Unabhängigkeit der Reaktionen von der Beschattungsdauer erhellt daraus, dass, *wenn* eine Reaktion erfolgte, sie beim Eintritt des Schattens in die Siphonalregion sofort erfolgte (Tab. II).

Tabelle II zeigt die Ergebnisse einer Versuchsreihe, gewonnen an 8 Muscheln der Art *A. cygnea*.

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[2] B. RAWITZ (1890–1892), W. A. NAGEL (1897), R. HESSE (1900), E. ZUGMAYER (1904), F. H. WEBER (1908).

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