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 frequency 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 pressoché 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

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To test these alternatives, embryonic organ rudiments were grown on a culture medium in which an extract from adult fowl heart tissue was substituted 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 and the maintenance of structure of adult and of juvenile 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 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. 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 SIF filter.

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

Results. The mesoblast of the 4-day chick limb-bud consisted, at the time of explantation, of structurally non-differentiated mesenchyme. During 3 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.

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 tissue derivatives from the culture medium. It should, therefore, be assumed that the metabolic requirements of the cells are satisfied by the

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