



Fig. 1. Ribonucléase oxydée.

Fig. 2. Ribonucléase réduite.

Action de la ribonucléase sur les cellules du carcinome d'Ehrlich (coloration d'Unna).

Durée d'incubation: 20 min.

Soulignons que ces phénomènes s'observent pour des doses de ribonucléase réduite excessivement faibles tandis que, même à concentration élevée, la ribonucléase oxydée est sans effet sur les cellules étudiées ici.

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#### Summary

The reduced form of ribonuclease acts on the cells of Ehrlich's carcinoma by producing important changes in the RNA content of cells:

- (1) First, there is an important synthesis of intracellular RNA and an accumulation of free nucleotides (from the external medium). This phenomenon accompanies the necrosis of the cells.
- (2) After this first stage begins a rapid degradation of intracellular RNA.

Simultaneously, the respiration of cells decreases whilst protein content remains unchanged.

The oxidized form of ribonuclease has no action on the cells.

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# Production of Pseudotumors in *Drosophila*, after Injection of Haemolymph

Recently Oftedal¹ has shown that the blood cells are the active elements in the formation of pseudotumors² in Drosophila. He determined the stage of larval development when the first changes in the normal cells take place. These blood cells undergo a progressive process of melanization, and become able to aggregate into clumps of different size. Finally, they appear as spindle-shaped elements with no evident structure of the original cells. Besides, there is no connection between the neighbouring organs and these melanotic masses, which are often floating in the hemocoele. Accordingly, we devised a method to test the participation of the haemolymph in the production of pseudotumors by reciprocal injections between tumorless and tumor stocks.

The usual microinjection technique with thin glass needles was used. The following experiments were carried out:

- (1) injections of physiological solution (NaCl at  $8^{9}/_{00}$ ) into larvae of a tumorless stock;
- (2) injections of haemolymph from two stocks with a different incidence of tumors into a normal stock;
  - <sup>1</sup> P. Oftedal, Z. Vererbungslehre 85, 408 (1953).
- <sup>2</sup> The melanotic masses are here referred to as pseudotumors (see Barigozzi<sup>3</sup>), and "tumor" is used only as an abbreviation.
  - <sup>3</sup> C. Barigozzi, Proc. IX. Int. Congr. Genetics (in press).

	In- jected larvae	Hatched flies	With- out tumor	tu-	% 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	4	5	55-55
Haemolymph of simu- lans (0%) in Varese	122	(3.16%)	5	0	0
(0%)	122	(4.09%)	3	U	U

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<sup>1</sup>).

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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#### 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

<sup>&</sup>lt;sup>1</sup> C. Barigozzi, Proc. IX Int. Congr. Genetics (in press).