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**The acquisition of a differentiated phenotype in  
sympathetic cells of chick embryo in vitro**

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**Embriologia e morfogenesi.** — *The acquisition of a differentiated phenotype in sympathetic cells of chick embryo in vitro.* Nota di GIANFRANCO TAJANA, MARCELLO MAROTTA e ANNAMARIA GUIGLIUCCI, presentata (\*) dal Corrisp. G. CHIEFFI.

RIASSUNTO. — È stato coltivato il territorio presuntivo di migrazione compreso tra i somiti 18–24 nell'embrione di pollo. Le cellule costitutive questo territorio rispondono al trattamento con NGF suggerendo la possibilità che in questi distretti embrionali esistano simpaticoblasti già determinati nel loro destino evolutivo.

The differentiation of sympathetic cells in vivo requires specific interactions between sympathetic neuroblasts and the associated mesenchyme (Norr 1973). It has been shown that it is possible *in vitro* to switch from an adrenergic to a cholinergic metabolic pathway by using conditioned media (Patterson 1977). Specific substances such as NGF can modulate the differentiation of presumptive chromoaffin cells in chick embryo (Aloe 1979). We asked whether the ability to respond to these specific stimuli is already present in migrating simpaticoblasts or is only acquired when these cells have reached their definite location in the embryo. We show here that migrating simpaticoblasts which will constitute the adrenal medulla of chick embryo are responsive to the NGF in a very early stage.

#### MATERIALS AND METHODS.

White Leghorn chick embryos, at a stage between 17 and 18 (Hamilton) corresponding to 52–72 hours of incubation and 30–36 somites, were used. The mesenchyme at the level from the 18th to the 24th somite was explanted and cultured for 5 days either in the presence or in the absence of NGF. Cultures were prepared according to the method of Fell. The medium consisted of equal amounts of 9-day chick embryo extract and chicken plasma. NGF, if present, was at a concentration of 50 ng/culture. Cultures were monitored daily and finally processed either for light microscopy or for fluorescence microscopy.

#### RESULTS

Cultures were scored, at 5 days, for the presence or absence of a "halo" effect. In the presence of NGF there was a dramatic increase in the neuritic growth in culture (Table I).

(\*) Nella seduta del 26 giugno 1980.

TABLE I.

N. EXPLANTS	NEGATIVE	POSITIVE	UNCERTAIN
125 (-NGF) . . . . .	109	2	14
125 (+NGF) . . . . .	52	73	—

Negative results (i.e. absence of the "halo" when NGF was present) were possible due to low yield of simpaticoblast in the explant. In Pl. I a typical culture in the absence of NGF is shown. Neuritic growth was almost completely absent. There was instead overgrowth of fibroblasts which migrated in a gel matrix consisting of polysaccharides.

In the presence of NGF (Pl. II, *a* and *b*) a remarkable "halo" effect could be seen. Long and thin processes irradiated from the explant indicating the organization of cytoskeletal elements. The growth of fibroblasts was very limited. The presence of biogenic amines could be clearly demonstrated by fluorescence microscopy (data not shown).

#### CONCLUSIONS

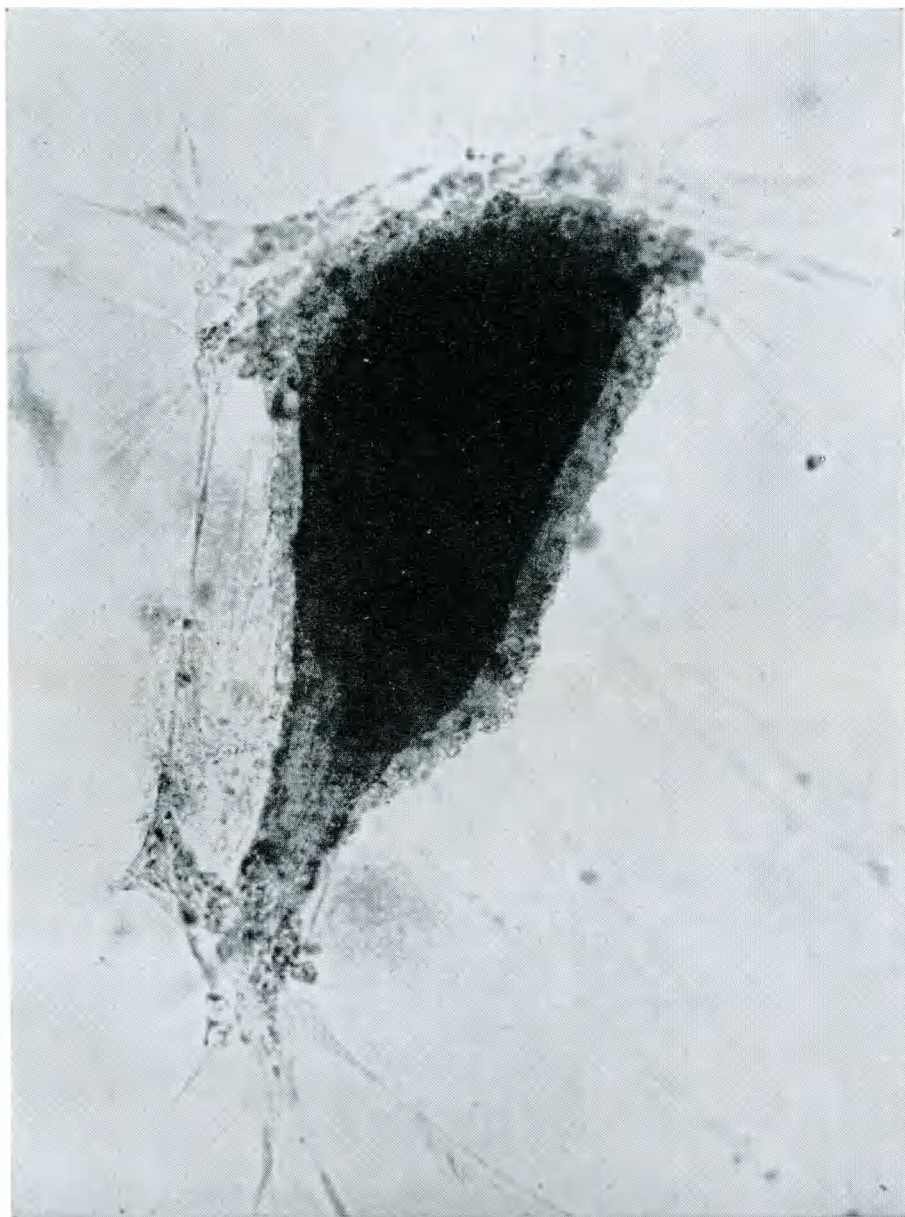
It is possible to isolate from chick embryos at a stage between 17-18 presumptive territories that will give rise to the medulla component of the adrenal gland. Simpaticoblasts are present in these explants. Our data clearly show that NGF is able to induce the differentiation of simpaticoblasts in culture. The acquisition of a differentiated phenotype is evidenced by the reorganization of the cytoskeleton and the resulting appearance of a very large number of neuritic processes. The response to NGF is very similar to that shown in other systems (Aloe 1979).

Furthermore the explants cultured in the presence of NGF showed a fluorescence typical of sympathetic cells, which suggests that biogenic amines are being synthesized and that also the simpaticoblasts may be functionally differentiated.

The tentative conclusion drawn from our work is that the ability to respond to specific "stimuli" that will induce differentiation is an intrinsic property of sympathetic cells.

#### REFERENCES

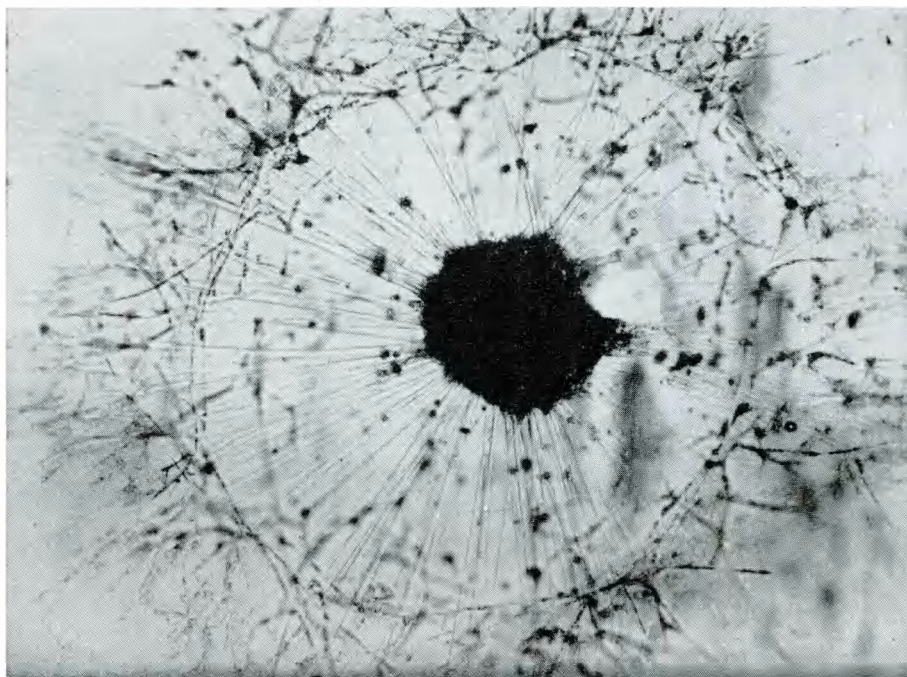
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