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Effects of various media on tissular and cellular structures of the superior cervical ganglion of the rat

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Summary. The superior cervical ganglia of the rat have been incubated in vitro for 1 h in basal medium Eagle (BME) with Hanks' salts, BME with Earle's salts, Krebs' solution and NCTC 109 medium. Comparison of the cell areas, established by a semi-automatic quantitative method, shows that the three former induce a 30–35% neuronal retraction, whereas NCTC 109 has no effect. Thus this latter medium seems the best one for studies using incubation of these cells.

In vitro preparations of the superior cervical ganglion (SCG) in different species and especially in the rat are widely used for electrophysiological, biochemical or pharmacological studies dealing with sympathetic nervous system. We have made, for example, studies about metabolism of sympathetic ganglia[2,3], different mechanisms of noradrenaline synthesis[4], synaptic transmission[5] and the functions of adenosine 3′-5′-cyclic-monophosphate (cyclic AMP) and its analogs[6,7]. These in vitro preparations have generally been done with Eagle medium with or without newborn calf serum and bicarbonate buffered Krebs' solution.

A major problem for all in vitro systems is the search for a physical and biochemical medium that optimizes neural cell performances[8]. The purpose of this work is to compare rat's SCG incubated in vitro, for 1 h, in 4 different media: basal medium Eagle (BME) with Earle's salts, BME with Hanks' salts, Krebs' solution, and NCTC 109 medium in order to select the best in vitro system.

Materials and methods. Left and right SCG were removed from 20 Wistar adult rats weighing between 230 and 400 g under Nembutal (40 mg/kg) i.p. anesthesia. The animals were divided into 2 groups. Group 1: in 5 rats, both SCG were dissected out, then treated by immersion fixation without previous treatment; group 2: in 15 rats, both SCG were dissected out and before immersion fixation were maintained into various media for 1 h at 36–36.5 °C (5% CO_2 and 95% O_2).

We have used 4 media: Krebs' solution, BME with Earle's salts, BME with Hanks' salts and NCTC 109 medium. The 3 latter media were used with or without 20% newborn calf serum.

After removal, each ganglion (incubated or not) was fixed in 2.5% glutaraldehyde in 0.177 M monodisodic phosphate buffer (pH 7.35) for 1 h at 4 °C. Then, they were postfixed in a 2% osmium tetroxyde solution (pH 7.4) for 1 h at 4 °C, dehydrated with acetone and embedded in Epon. Each ganglion was cut with a Reichert OMU 3 microtome and sections were contrasted with uranyl acetate and lead citrate and examined in a Siemens (Elmiskop 101) electron microscope. In each group, 1 SCG was taken at random. Semi-thin sections are made and photographied in a contrast microscope.

The pictures (magnification × 560) were used to measure cellular areas; 40 neuron areas were fixed in each group (incubated or not) by a system for semi-automatic quantitative evaluation of images (MOP/AMO 1 Kontron). We have made the calculations of mean area, SEM, SD and variance.

Results. Three morphological criteria were used to compare the efficacy of each solution: general aspect of the ganglia, size and ultrastructural morphology of neurons.

On phase contrast microscopical semi-thin sections: there are great differences between SCG morphology according to media. The most important modification in comparison with the control ganglia, fixed by immersion (figure 1), is a cellular retraction with a very high increase of intercellular spaces and the occurrence of dark neurons: especially observed in SCG incubated in the 2 BME media with newborn calf serum (figures 2 and 3) and in Krebs' solution

Neuronal area of SCG: comparison between SCG control and incubated for 1 h in different media*

	Controls	BME with Hanks' salts + SC	BME with Earle's salts + SC	Krebs' solution	NCTC 109 medium + SC
Area (μm^2) ± 95% SEM (n)	272.16 ± 2.31 (40)	192.19 ± 4.21 (40)	177.84 ± 4.40 (40)	189.27 ± 2.93 (40)	267.19 ± 3.68 (40)
Neuronal retraction (%)		30	35	30	2

SC = 20 per cent newborn calf serum; n = number of samples. * The neuronal area after fixation by perfusion is 318.68 ± 4.19 (40).

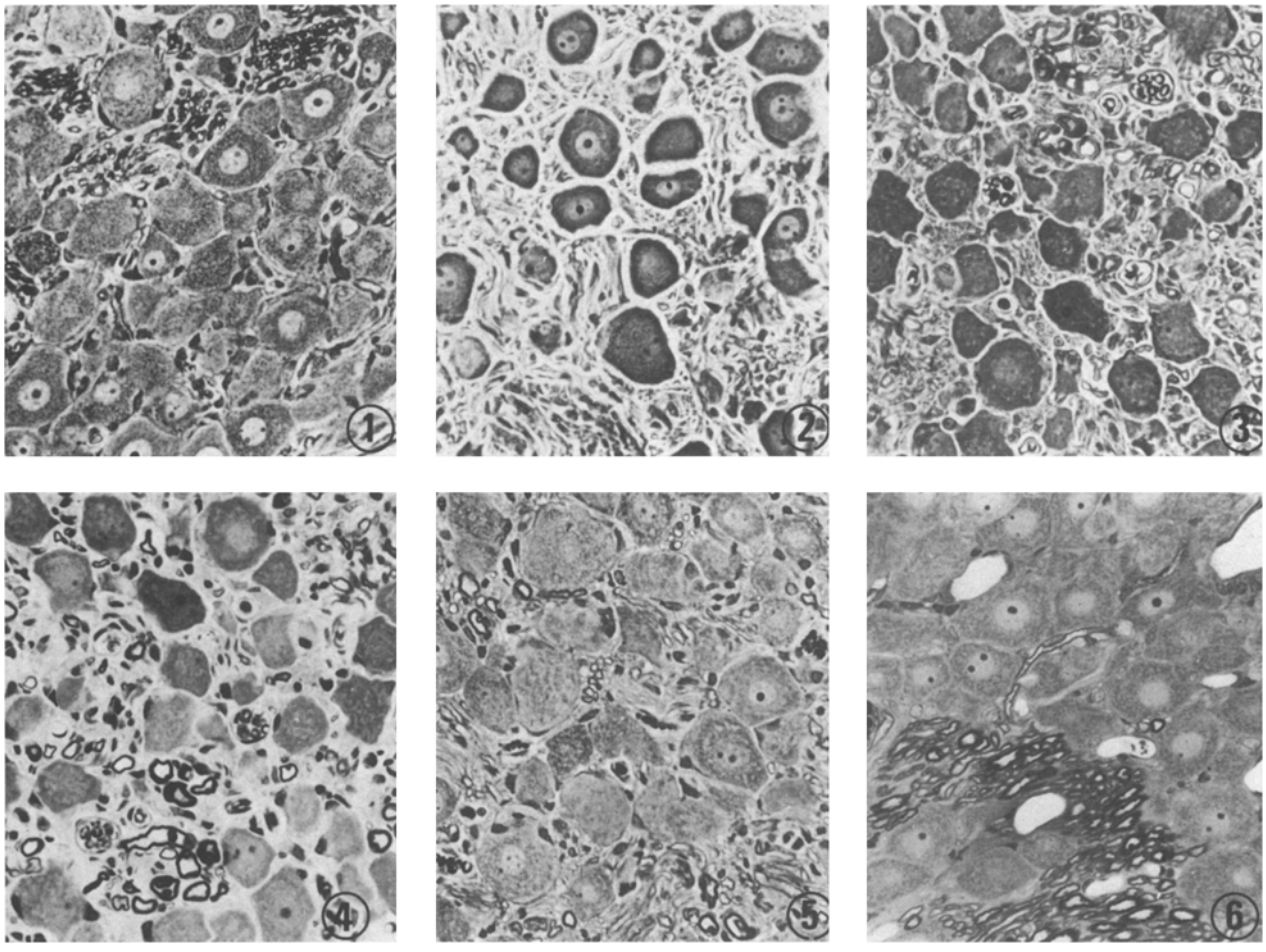

Fig. 1. SCG control (fixed by immersion). Fig. 2. SCG incubated in BME with Hanks' salts: cellular retraction and dark neurons. Fig. 3. SCG incubated in BME with Earle's salts: cellular retraction and dark neurons. Fig. 4. SCG incubated in Krebs' solution: cellular retraction and dark neurons. Fig. 5. SCG incubated in NCTC 109 medium: cellular retraction and dark neurons are absent and the tissue is quite similar to the control. Fig. 6. For comparison, aspects of the SCG fixed by perfusion. × 480.

(figure 4), whereas a quite similar morphology is observed in SCG incubated in NCTC 109 medium with newborn calf serum (figure 5). The value of neuronal areas corroborate the cellular retraction. Indeed, incubation into the 2 BME media and in Krebs' solution induces a 30–35% decrease of neuronal sizes, while this decrease is only 2% in the NCTC 109 (table).

On electron microscopic observations: 1. BME with newborn calf serum: we observed a very important oedema, lesions of most of the glial cells, sometimes completely destroyed. Cytological alterations predominate in myelinated fibres and in neuronal perikaryon, i.e., dictyosomes of the Golgi complex, endoplasmic reticulum, and mitochondria are enlarged and sometimes broken out. 2. Krebs' solution without newborn calf serum as employed in most biochemical or physiological studies. After incubation, we observed an important oedema, too, but myelinated fibres and neuron satellite cells are well conserved. In the sympathetic neurons, the cytoplasmic organelles are well preserved, although the Golgi apparatus and some mitochondria are lightly swollen. 3. NCTC 109 medium with newborn calf serum gives the best results. Intercellular spaces are quite normal and only few mitochondria, few reticulum endoplasmic cisternae and few Golgi areas are lightly enlarged.

Discussion. At each stage of fixation and embedding, changes are inevitably introduced: tissue dimensions are altered and molecular rearrangement occurs[9]. It is well known that conservation of tissular and cellular structures are obtained by perfusion (figure 6). Indeed, comparison of areas shows that after immersion fixation, the perikaryon of SCG neurons were retracted about 15% in regard to ganglia taken out from perfused animals (table).

In our experimental conditions, only immersion fixation has been used, thus we only refer to this procedure as control. The most striking fact is that a 30–35% neuronal retraction occurs in ganglia incubated in BME and Krebs' solution. On the contrary, incubation in NCTC 109 medium induces only a 2% decrease of neuronal size. From these morphological and ultrastructural studies, it is evident that the incubation in the 2 BME media involves more important structural alterations than the Krebs' solution incubation. Immersion fixed ganglia and NCTC 109 medium incubated ganglia have identical morphology and ultrastructure.

Numerous factors can more or less affect structures in incubated tissue. Many authors have shown that substrat and oxygen privation involves, in most cases, tissue and organ death when maintained in vitro[10].

In these experiments, anoxic factor does not interfer because oxygenation is constant during incubation. Moreover, it was confirmed that if there are great alterations, they do not occur especially into the central part of the ganglia. From a general point of view, Varon[8] has

shown that defined media (balanced salt solutions enriched with glucose, amino-acids and vitamins) fail to support neural cultures unless supplemented biological fluids (various fetal or adult sera, embryonic extracts). The role of these biological supplements is to provide not only greater nutritional support, but also some special agents necessary for the survival, proliferation and/or expression of differentiated properties of the cultured cells or organs.

In our experimental conditions, it appears that the 2 BME media involve important modifications of the SCG morphology, that Krebs' solution is less toxic because ultrastructure is conserved, and finally that the NCTC 109 with newborn calf serum permits the best conservation of the SCG structures.

In conclusion, these results show that NCTC 109 medium with 20% newborn calf serum could be used preferentially for the biochemical, electrophysiological and pharmacological analysis where the SCG must stay in artificial medium.

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Formation of lung colonies by mouse ascitic teratocarcinomas

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Summary. Intravenous transplantation of teratocarcinomas resulted in the formation of colonies only in the lung. The lung colony system would be advantageous for the quantitative studies of differentiation of teratocarcinoma stem cells.

Recently, mouse teratocarcinomas have been shown to be useful for studying embryonic development in mice[2]. But few quantitative studies have been reported. Williams and Till[3] have reported that virus-transformed rat embryonic cells form colonies in the lung when they were injected i.v. This method provided the basis for a quantitative assay for malignancy of transformed cell lines. Recently, we also induced colony formation of teratocarcinomas in the lung by i.v. injection of embryoid bodies (EBs)[4,5]. In this paper, we show that this method may be useful for the quantitative study of differentiation of teratocarcinoma stem cells.

Materials and methods. Embryoid bodies (teratocarcinoma OTT6050), serially transplanted into mice (129/Sv) every 3 weeks, were taken from the peritoneal cavity of the mouse and washed 3 times with phosphate buffered saline (PBS). EBs suspended in 0.25 ml of PBS were injected through the tail vein of syngeneic mice. At the same time, 0.25 ml aliquots taken from the same suspension were placed in culture dishes, dried and stained with Giemsa's solution for counting EBs in the dish, thereby estimating the number of EBs injected. For counting the number of colonies, the mice wer killed and their lungs were excised. These lungs were fixed in the Bouin's fixative for 1 day and were transferred into 70% alcohol. Colonies visible on the lung surface were counted.

Results and discussion. When EBs were injected through the tail vein, they formed teratomatous colonies in the lung (figure 1). These colonies could be identified on the surface of unfixed lung about 2 weeks after injection. When the lungs were fixed in the Bouin's fixative and thereafter dipped into 70% alcohol, even about 7-day colonies on the lung surface were visible with ease. In the experiment, surface colonies that could be seen only with the naked eye were counted. The teratomatous colonies have never been found on the surfaces of the other organs.

Colonies often contained embryonal carcinoma cells as well as various types of tissues and these colonies were transplantable (figure 2).

The number of colonies formed on the lung surface increased in proportion to the number of EBs injected

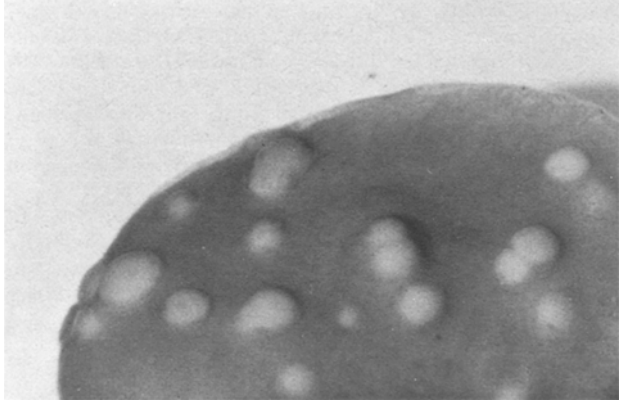

Fig. 1. The teratomatous colonies, which were derived from embryoid bodies, are seen on the surface of lung 20 days after injection. × 30.

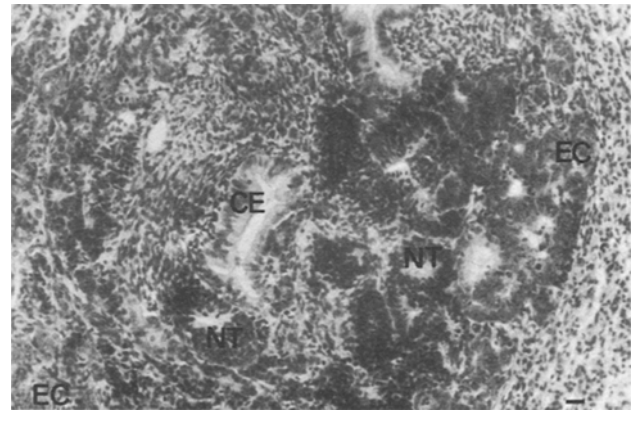

Fig. 2. The histological section of a teratomatous colony of 30 days old. EC, teratocarcinoma stem cells (embryonal carcinoma cells); NT, neural tube; CE, ciliated epithelia. Bar = 20 μm.