Dysdifferentiation of the Neural Crest Cells in the Suckling Mice

Some of the suckling mice injected with mitomycin C within 24 to 48 h after birth survived up to 9 to 13 days after birth, and in their histological observation the multiple hyperplasia or excessive cell proliferation and the heterotopic deposition of the melanin pigment or granule were often recognized.

Materials and methods. The mice of the ICR-JCL strain, a total of 370, were injected i.p. with a dose of 8 mg/kg within 24 to 48 h after birth and 88 survived up to the 9th day after birth. 30 of the 88, and 10 mice not treated with mitomycin C, were used for the histological observations. The specimens were fixed with 10% neutral formalin and embedded in paraffin. Transverse, frontal and sagittal sections of 7 μm in the thickness were prepared and stained with hematoxylin and eosin or ferric chloride stain for melanin.

Results and discussion. The multiple hyperplasia or excessive cell proliferation and the heterotopic deposition of the melanin granule or pigment were often observed in certain tissues of the same individual, and some of them resembled the histological findings in some tumors. They were classified roughly into three groups. A) Not only hyperplasia but heterotopic deposition of the melanin granule or pigment were present in the optical nerve, choridea, sclera, tooth germ, oral mucosa membrane, and gastric mucosa; B) Hyperplasia or excessive cell proliferation were seen in the periostium, perichondrium, bone, small blood vessels in the cranial parts, glands and ducts of oral cavity, stomach, intestine, alveolar ducts and branchioles of the lung, sympathic nerve plexus of the peritoneum and pleura, muscular tissues, and brain; C) Hyperplasia consisted of excessive by fine fibres seen in the cranio-spinal ganglions and nerves, and the pia-mater. Hyperplasia consisted of atypical cells such as the elongated spindle or cylinder-shaped cells, large irregular cells, and oval or round cells, and the ratio of the elongated spindle or cylinder-shaped cells was extremly high compared to other cells. Hyperplasia or excessive cell proliferation were often characterized by the interlacing pattern of the small streams of the above cells, especially the elongated spindle or cylinder shaped cells.

The function of mitomycin C against DNA in the bacteria or bacteria phage is related to the repair replication of the damaged DNA similar to the X-ray or ultraviolet action against the DNA in them[1,2]. It is said that tumors from different sources have been found to respond differently to mitomycin C and that this can be explained by the difference in the ability of the tumor cell to repair the damaged DNA[1]. It was found in our laboratory that the undifferentiated neural crest cells in mice embryos showed a specific sensitivity to mitomycin C and the DNA and protein in the neural crest cells denatured and disappeared[3], and that further the DNA and protein in them denatured easily by heat or acid[4]. It is thought that these phenomena may be related to the specific mechanism of the repaor replication of the damaged DNA[3], and to the specific sequence of DNA nucleotides and protein synthesis in them. On investigation, by making the best of the specific sensitivity of the undifferentiated neural crest cell to mitomycin C in the prenatal or postnatal development, it was found that the undifferentiated neural crest cells may be present, scattered in certain sites [5,6]. In the suckling mice which survived up to the 9th day after birth, the neural crest cells, which may be of spindle or irregular shape[7–10] and

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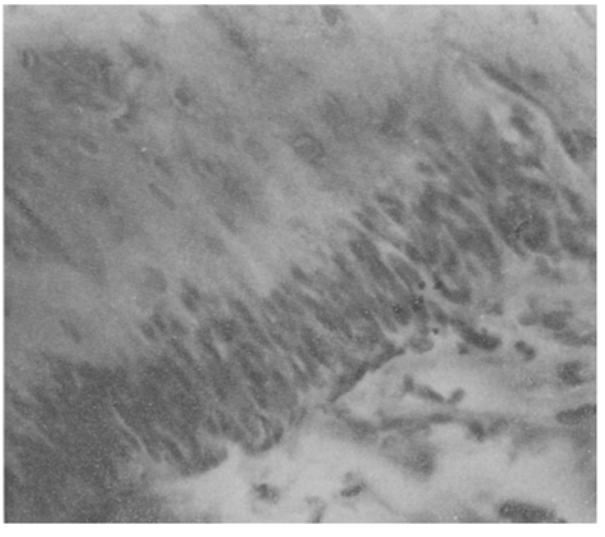

Fig. 1. Hyperplasia in the bone and periosteum. H-E stain. ×600.

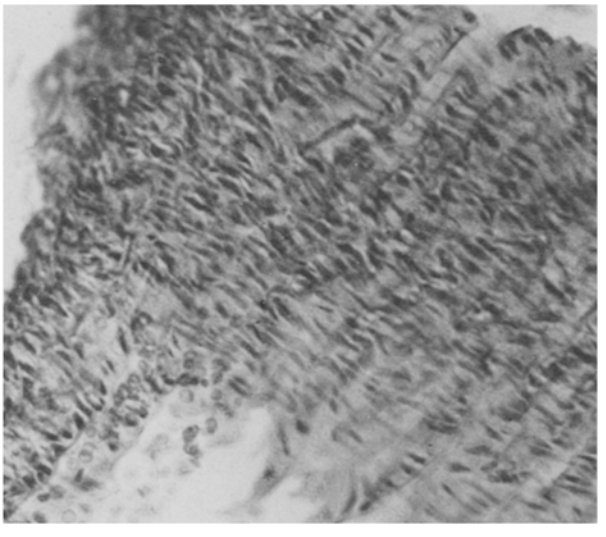

Fig. 2. Hyperplasia in the peritoneum. H-E stain. ×600.

denature by mitomycin C, were hardly seen in the above sites, and hyperplasia or excessive cell proliferation were present. It is speculated that the inherited multi-systemic diseases may be related to the cells derived from the same origin, especially the neural crest[6,11]. On the other hand, it is said that the cells from patients with the hereditary disease, xeroderma pigmentosum, bring about mutation such that the repair replication of DNA is either absent or much reduced, and patients with xeroderma pigmentosum develop fetal skin cancers when exposed to sunlight, and so the failure of the DNA to repair in the skin must be related to carcinogenesis[12]. It is speculated that the hyperplasia or excessive cell proliferation and the heterotopic deposition might occur by the dysdifferentiation[13] of the neural crest cells.

Zusammenfassung. Nachweis, dass bei Entwicklungsstadien von Mäusesäuglingen, die mit Mitomycin C injiziert wurden, Hyperplasien mit Melanin-Ablagerungen auftraten, was mit «Dysdifferenzierungen» von Zellen der Neuralleisten zusammenhängen dürfte.

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Histophotometric Measurements of the DNA Content in the Ovarian Follicle Cells of *Lacerta sicula* Raf.[1]

In *Lacerta sicula*[2], in the ovarian follicles ranging from about 100 to 1500 µm in diameter, the follicular epithelium consists of 3 different types of cells (Figure 1). Outstanding are the 'pyriform cells', with enlarged body containing a large nucleus and the apex toward the oocyte. These cells represent the only clear example in vertebrates so far studied by electron microscopy, of cells in direct connection with the oocyte through intercellular bridges[2-6].

By electron microscopy Taddei[6] and Taddei and Barsacchi-Pilone[7], with cytological, cytochemical and autoradiographic methods, have observed that, in contrast to the first phase of oocyte growth characterized by a monolayered follicular epithelium, typical nucleoli are lacking in the germinal vesicle after differentiation of the pyriform cells and incorporation of ^{3}H uridine, very high in these cells, is absent in the oocyte nucleus[6,7]. Taddei[6] has suggested that, at this stage, together with other materials, ribosomes are synthesized by the pyriform cells and transferred to the oocyte through intercellular bridges, so that these cells, by constituting a highly integrated system with the oocyte, seem to function as 'nurse cells'.

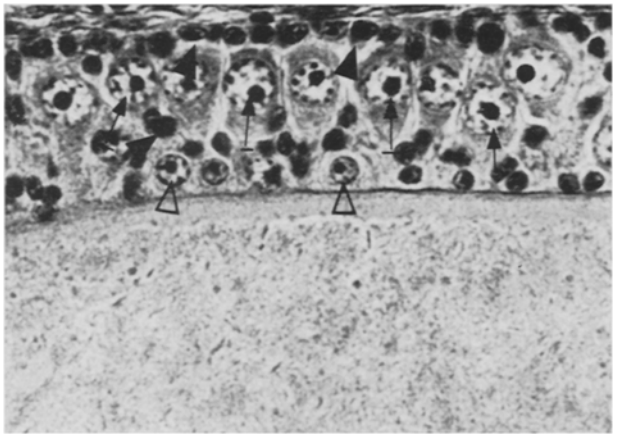

Fig. 1. Light micrograph of the polymorphic follicular epithelium of *Lacerta sicula*. Note the nuclear characteristics of the three types of follicular cells: small follicular cells (black arrow), pyriform cells (arrow with bar), intermediate cells (white arrow) (450×).

Nurse cells are described in the oogenesis of some invertebrates; especially in the meroistic insect ovary, the function of these cells of synthesizing and transferring to the growing oocyte RNA through intercellular bridges is well-documented; it has further been observed that during differentiation of these cells, the nucleus undergoes endomitotic polyploidization[8-10].

The present work investigates whether the differentiation of the lizard's pyriform cells is accompanied by a process of polyploidization, with a view to better understanding of the function of these cells in the oogenesis of *Lacerta sicula*.

Materials and methods. For the histophotometric determination, oocytes of the lizard *Lacerta s. sicula* Raf., taken in November, were manually removed from their connectival theca under the dissecting microscope using watchmaker's forceps. The oocytes surrounded by the follicular epithelium were fixed in ethanol-acetic acid (3:1) and transferred into 45% acetic acid on a slide previously coated with gelatin (0.1%) chrome alum (0.001%) solution, covered with a siliconized cover slip and then squashed. The preparations were frozen on dry ice and the cover slip removed with a razor blade[11].

After 5 min in 95% alcohol, the Feulgen reaction was carried out on the air-dried slides. After hydrolysis in 1 *N* HCl (12 min at a constant temperature of 60 °C.), the slides were stained for 90 min with Schiff's reagent and rinsed in 3 baths of freshly-prepared SO_2 solution.

[1] This research was supported by a C.N.R. grant.
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