Multiple oncogenesis of neural crest cells by steroids in suckling mice

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Summary. In suckling mice injected with steroids, multiple tumors occurred in the sites where neural crest cells normally are present. It is speculated that the phase of the cell cycle of the neural crest cells may have something to do with its cell surface and cellular phenotypic expression in the system mediated by cyclic AMP.

Steroids such as hydrocortisone increase the cyclic AMP and cell death appears to be mediated by cyclic AMP[1,2]. It is thought that cell death by cytotoxic action correlates with mutation[3]. The neural crest cells of mouse embryos, whose mother had been injected i.p. with hydrocortisone, show cell death. The same is true in neural crest cells treated with alkylating agents[4]. We consider that especially the neural crest cells may have something to do with cyclic nucleotides[4,5]. Therefore, an investigation was undertaken to discover whether tumors of neural crest derivatives could be induced by the injection of steroids into newborn mice.

Materials and methods. Hydrocortisone and androgen were used as steroid. Saline suspension of cortone acetate (acetate cortisone 25 mg/ml, Merck, Rahway, N.J., USA) was used as hydrocortisone. Androgendepot (enanthic acid testerone 50 mg/ml, capric acid testerone 50 mg/ml, Yamanouchi Seiyaku, Japan) was used as androgen. Within 24 h after birth, 50 mice of the ICR-JCL strain were injected i.p. with a dose of 35 mg/kg of hydrocortisone and 10 mice were injected i.p. with a lower dose. Similarly, 40 mice were treated with a dose of 670 mg/kg of androgen within 24 h after birth, and 10 mice were given a lower dose. The animals were sacrificed after 1–6 days following injection. 50 individuals of comparable age were used as control specimens. All specimens were fixed in 10% neutral formalin and embedded in paraffin. Transverse, frontal and sagittal sections of 7 μm in thickness were prepared serially, and stained with hematoxylin and eosin or ferric chloride stain for melanine.

Results and discussion. In suckling mice treated with a dose of 35 mg/kg of hydrocortisone, or with a dose of 670 mg/kg of androgen, various morphological and histological abnormalities were recognized. Abnormalities were similar to those observed in suckling mice injected with mitomycin C[6–8], or endotoxin[9]. Furthermore, in the present experiment hardly any differences were noted between the effects of cortisone and androgen. Both treatments resulted in retarded physical development. Characteristic features of abnormal development from 1 to 6 days after treatment were the following: Dry or rough skin; marked shortening of the trunk with relatively less shortening of the extremities; knock-knees; deficient hair formation in the trunk region; akinesis or rigidity; fine and irregular tremor of outstretched extremities. On histological observation, multiple tumors or excessive cell proliferation were seen in many different locations, all of which, however, are known to become colonized in normal development by neural crest derivatives. Tumors were seen in periostium, perichondrium, bone marrow, the

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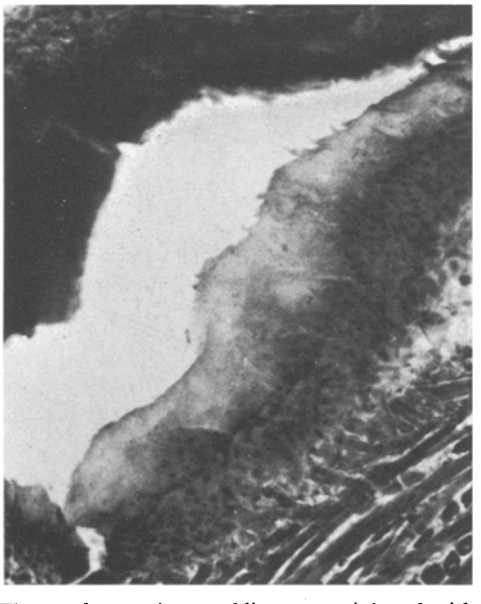

Fig. 1. The oropharynx in a suckling mouse injected with hydrocortisone. H–E stain. ×800.

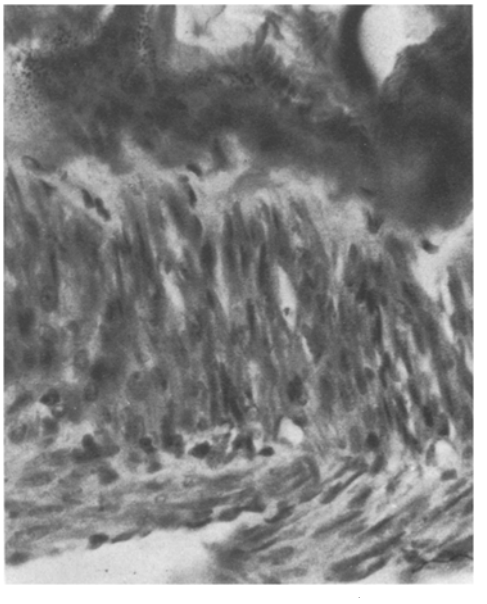

Fig. 2. The stomach in a suckling mouse injected with hydrocortisone. H–E stain. ×800.

walls of the blood vessels, heart, spleen, parotid glands, submandibular glands, teeth, oral mucosa, stomach, intestine, liver, lungs, pancreas, adrenal glands, thyroid gland, thymus, vibrissae, skin, eyes, cranio-spinal nerves and ganglia, sympathetic nerves and ganglia, nerve plexus of the periostium, pleura, pia mater, brain, kidneys, uterus, and testis. Tumors were composed of spindle-shaped cells, elongated spindle-shaped cells, large irregularly shaped cells, or of basophilic round or oval cells in various sizes. In the above tissues and organs, spindle-shaped cells refractory to the stains were present. In some cases the tumors formed cell masses or cell groups, for example in the islets of Langerhans of the pancreas, in the medulla of the adrenal glands, and in the glomeruli of the kidneys. In other cases, tumors showed the characteristics of the interlacing network of wavy, flowing streams of spindle-shaped cells in which nuclear palisading was seen, for instance in the submucosa, muscularis mucosae, smooth muscle layers or serosa of the stomach and intestine. Tumors of melanoma type were seen in the oral mucosa, tongue, vibrissae and skin. Further, heterotopic melanin pigmentation and hyperkeratinization were seen, for example in the gastric mucosa. With increasing age, tumors developed and erosions were seen. In mice injected with lower doses, morphological abnormalities and tumorous growths were generally absent.

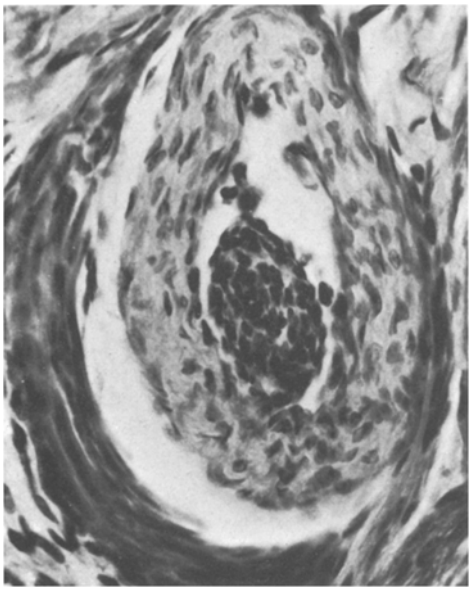

Fig. 3. The vibrissae in a suckling mouse injected with androgen. H–E stain. ×800.

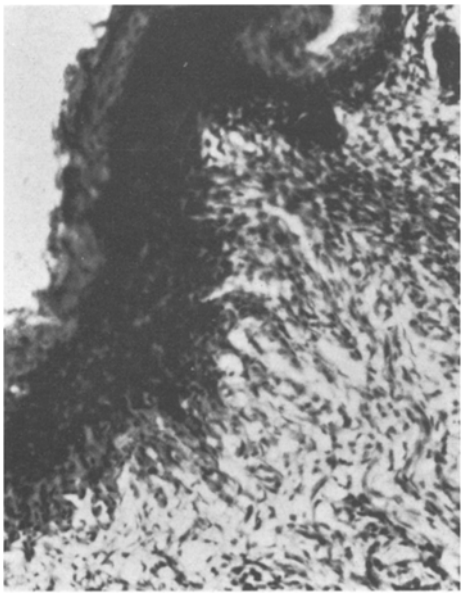

Fig. 4. The skin of the orofacial region in a suckling mouse injected with androgen. H–E stain. ×400.

It is now well-established that an adenyl cyclase at the cell surface is stimulated by a wide variety of steroid hormones and that thereby cyclic AMP concentration increases. Cell death is mediated by cycle AMP[1,2], and cell death by cytotoxic action correlates with mutation, and lethal damages to DNA are manifestations of the same phenomena[3]. We consider that especially the neural crest cells may have something to do with cyclic AMP[4,5] and that their DNA nucleotides sequences may be specific[4]. It is said that cyclic AMP has a direct effect upon transcription, but not upon replication of DNA[10]. It has become a question whether so-called DNA repair processes restore the original DNA base sequence or only restore the DNA template sufficiently for DNA replication to occur and for the cell to survive, but with its DNA still modified so as to lead to subsequent base sequence errors during DNA synthesis and hence to mutation. It has been stated that cyclic AMP can induce undifferentiated cells to differentiate into the derivatives of the neural crest[11–13]. We consider that the neural crest cells may be undifferentiated and pluripotential cells[14,15]. It was ascertained[16] that the regional distribution of the neural crest cells in the suckling mice[14,15] corresponds to their distribution based on Pearse's hypothesis[17,18]. Further, it was already reported that multiple tumors in suckling mice injected with mitomycin C may be brought out by dys- or disdifferentiation of neural crest cells[6], that multiple tumors in suckling mice injected with endotoxin may somehow be connected with cyclic AMP in the neural crest cells[9], and that multi-systemic diseases may be related to neural crest cells[7,15]. It has been suggested that the sensitivity of the neural crest cells may be related to cell cycle[19], that cell death in synchronized cells follows arrest in the G_1 phase of the cell cycle[1], and that cyclic AMP may act in the G_1 phase[20]. From the above, we speculate as follows: The phase of the cell cycle in the neural crest cells may have an important relation to cell surface properties and thus to cellular phenotypic expressions in the system mediated by cyclic AMP. Errors may occur during replication and/or transcription of neural crest DNA, induced by steroids and mediated through cyclic AMP, so that the neural crest cells may mutate or denature. We suggest that a similar process may account for the action of mitomycin C[6–8,19] or endotoxin[9] in suckling mice.

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