EMBRYONIC RHABDOMYOBLASTOMAS INDUCED BY METHYLNITROSOUREA IN THE RETROBUCCAL POUCH OF SYRIAN HAMSTERS

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The carcinogenic action of methylnitrosourea was studied on the mucous membrane of the retrobuccal pouch of 60 Syrian hamsters. The neoplasm developed in various situations in 20 of the 25 animals surviving until the time of appearance of the first tumor. Neoplasms developed at the site of injection (in the left retrobuccal pouch) in 13 hamsters (52%) while in 11 animals (44%) tumors appeared in the stomach. Twelve neoplasms of the retrobuccal pouch were classed by the writers as embryonic rhabdomyoblastomas of varied histological structure.

Spontaneous tumors of the retrobuccal pouch of Syrian hamsters are exceptionally rare: only one case of fibromyxosarcoma has been published [6]. Induced tumors were first obtained by Salley [7] after administration of carcinogenic polycyclic hydrocarbons into the retrobuccal pouch. No information on the results of administration of nitroso compounds into the retrobuccal pouch of Syrian hamsters could be found in the accessible literature.

The experimental production of embryonic rhabdomyoblastomas would help to shed light on the morphology and histogenesis of these rare and not easily diagnosed tumors.

The present investigation is part of a general study of the carcinogenic action of dimethylnitrosamine (DMNA), diethylnitrosamine (DENA), and methylnitrosourea (MNU) on different animals when administered in different ways [1, 4].

EXPERIMENTAL METHOD

Experiments were carried out on 60 Syrian hamsters of both sexes aged 1-2 months. The largest group of 40 animals received MNU, in view of the marked local carcinogenic action on this compound. DMNA, DENA, and MNU were injected by means of a tuberculin syringe with a bent needle into the left retrobuccal pouch twice a week for 9 months in a dose of 0.1 mg of each compound per injection. The maximal total dose of each compound was 7.5 mg per animal (62 mg/kg), or about 1.5 LD_{50} for rats. The control group of 110 hamsters of the same breed received no experimental treatment and were kept simultaneously and under the same conditions. All the experimental and control animals remained under observation until natural death (about 1.5 years). Material for histological investigation was fixed in 10% acid formalin solution and embedded in paraffin wax and celloidin. Sections were stained with hematoxylin and eosin, with picrofuchsin mixture, toluidine blue, Heidenhain's iron hematoxylin, with silver by Gomori's method, or by the PAS reaction, and frozen sections were stained with Sudan III.

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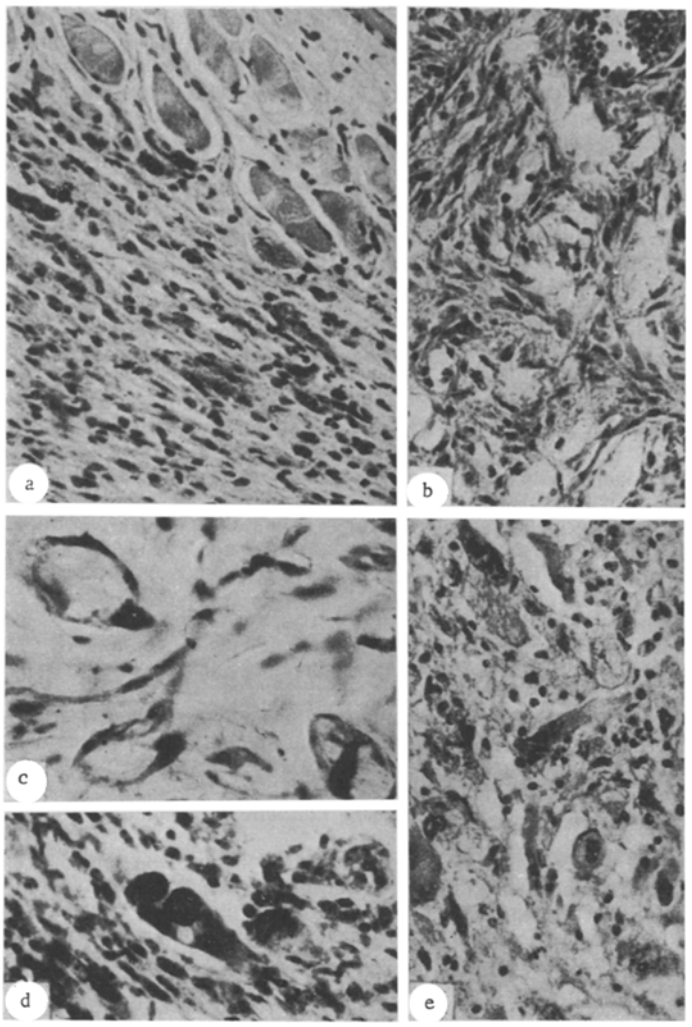

Fig. 1. Morphological varieties of embryonic rhabdomyoblastomas (stained with hematoxylin and eosin): a) compact type (120×); b) myxoid type: tumor cells arranged in a syncytium (250×); c) mixed type: ring-shaped cells in a myxoid area (500×); d) mixed type: rocket-shaped cell (500×); e) polymorphocellular variant: giant cells and symplasts (250×).

EXPERIMENTAL RESULTS

After injection of DMNA tumors (hepatomas) developed in five of the six animals which survived until the time of appearance of the first tumor (25 weeks from the beginning of the experiment). No tumors developed in the hamsters receiving DENA. The negative results were evidently due to the ineffective dose of DENA by the chosen mode of administration.

The group of animals receiving MNU was of the greatest interest. Tumors appeared in different situations in 20 of the 25 animals surviving until the appearance of the first tumor (31 weeks). Tumors developed in the left retrobuccal pouch, at the site of injection of MNU, in 13 hamsters (52%), while in another 11 animals (44%) neoplasms were found in the stomach (three papillomas of the forestomach, one glandular polyp, one leiomyoma, and six tumors of the neurofibrosarcoma type), probably as the result of the entry of the compound into the gastro-intestinal tract. In seven cases tumors of the retrobuccal pouch were combined with other neoplasms: of the stomach (four cases), of the skin and adrenal (one case each); in one female which died 72 weeks after the beginning of the experiment, seven tumors were found: two tumors in the retrobuccal pouch, two in the skin, and one each in the liver, stomach, and ovary. In the ex-

periment with MNU, 20 animals thus developed 34 tumors, and one hamster also developed reticulosis. Comparison of the results of this group of experiments with those obtained in the control animals (only two tumors – one carcinoma of the adrenal cortex and one case of papillomatosis of the forestomach, developing after 59 and 92 weeks respectively) suggests that the overwhelming majority of tumors appearing after administration of MNU and, above all, the tumors developing in the retrobuccal pouch were induced.

Of 17 tumors of the retrobuccal pouch, 12 were identified as embryonic rhabdomyoblastomas. The external appearance of these tumors was fairly typical: soft, hemispherical structures from 0.1 to 1.5 cm in diameter, reddish or yellowish in color, frequently with an ulcerated surface. The tumor was located more frequently in the middle part of the pouch, less frequently in the intermediate fold, and was intimately connected with the muscular coat without invading the surrounding tissues. No metastases were found.

Histological Investigation. Tumor cells infiltrated the whole wall of the pouch diffusely from the subepithelial connective tissue to the muscular coat, spreading into the space between the muscle bundles. The main mass of the tumor consisted of small, round, elongated or band-shaped cells (Fig. 1a) with fine eosinophilic granules in the cytoplasm, resembling embryonic rhabdomyoblasts [2, 5, 8]. The cells formed a syncytium which was seen particularly clearly in tumors rich in intercellular substance (Fig. 1b). Large ring-shaped cells (because of the presence of vacuoles), spider-shaped or rocket-shaped cells (Fig. 1c, d), as well as individual malformed symplasts (Fig. 1e) were seen. The nuclei varied in size and shape: vesicular, compact, sometimes with distinct nucleoli, and containing a reticular or granular chromatin. Solitary mitoses were present. The cell cytoplasm stained with picric acid and contained neutral and acid nonsulfonated (giving β-metachromasia) polysaccharides, glycogen granules, and small quantities of sudanophilic lipids. No lipids were found in the vacuoles. These places usually stained only weakly with picric acid and acid nonsulfonated polysaccharides were present in larger amounts.

Uniform hyaline-like areas were seen in the cytoplasm of some cells.

Cross-striation was rarely seen (20-45% of cases) in the embryonic rhabdomyoblastomas, and then only in single cells, so that its absence does not rule out a diagnosis of this tumor [3].

Indistinct cross-striation was observed in only one of the cases studied.

A characteristic feature of the tumor was the presence of distinct argyrophilic cuffs around the tumor cells, emphasizing the cell boundaries even in areas of necrosis. This feature is common to all tumors of myogenic origin.

The stroma of the neoplasms was usually well-marked. The ground substance contained small quantities of collagen material, together with separate thin argyrophilic fibrils; acid nonsulfonated and neutral polysaccharides were detected.

Depending on the predominant type of cell and the quantity of stroma, the following types of embryonic rhabdomyoblastomas can be distinguished: myxoid, compact (small- and spindle-cell), polymorphocellular, and mixed.

In the present experiments their frequency was about equal. In two hamsters, two tumors of different types were discovered.

Large areas of the tumor (irrespective of its type) were necrotic, and diffuse infiltration by neutrophils was seen. There were many thin-walled vessels, so that some of the tumors resembled a capillary hemangioma. Sometimes small foci of hyalinosis were found. In peripheral zones of the tumor and at the boundary with the surrounding tissues edema was usually observed, together with many mast cells with scattered heparin granules. Preexisting muscle fibers, located among the tumor cells, were in a state of degeneration and necrobiosis (swelling, myolysis, granular degeneration, loss of cross-striation and of nuclei). In the boundary zones there were muscle bundles showing amitotic division of the nuclei, and forming conglomerations and chains.

A possible histogenetic source of the embryonic rhabdomyoblastomas, in the writers' view, would be foci of regeneration of cross-striated muscle fibers in the wall of the retrobuccal pouch of the hamster.

Penetration of the carcinogen into the substance of the pouch may result from defects in the epithelial cover, arising either spontaneously or through the action of MNU.

Diffusion of an aqueous solution of the nitroso compound through the undamaged wall likewise cannot be ruled out.

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