Effect of 5-Azacytidine on Developing Mouse Embryo

5-Azacytidine possesses remarkable antileukemic and bacteriostatic[1,2] properties. Recently, it has been found that 5-azacytidine is capable of provoking interruption of pregnancy in mice[3]; for this reason it seemed of interest to investigate its cytological effects on developing mouse embryo using histology and autoradiography with labelled nucleic acids precursors.

5-Azacytidine and 5-azacytidine-4-^{14}C (4.1 μC/μmol) were synthesized[4] by Dr. Pískala and Dr. J. Morávek, both of this Institute. Female mice were used throughout the experiment. All of them were of random bred strain H, kept under standard conditions. The mice with vaginal plugs were isolated and regarded as zero day pregnant. In one instance (histological examination of the embryonal nervous tissue on 12th day of gestation) ovulation was synchronized by administration of combined serum and chorionic gonadotropins (Gestyl. Organon, Holland and Praedyn, Léčiva, Czechoslovakia). Conventional histological sections were processed to autoradiography (Kodak AR.10 stripping film). Total nucleic acids were extracted with 5% trichloracetic acid.

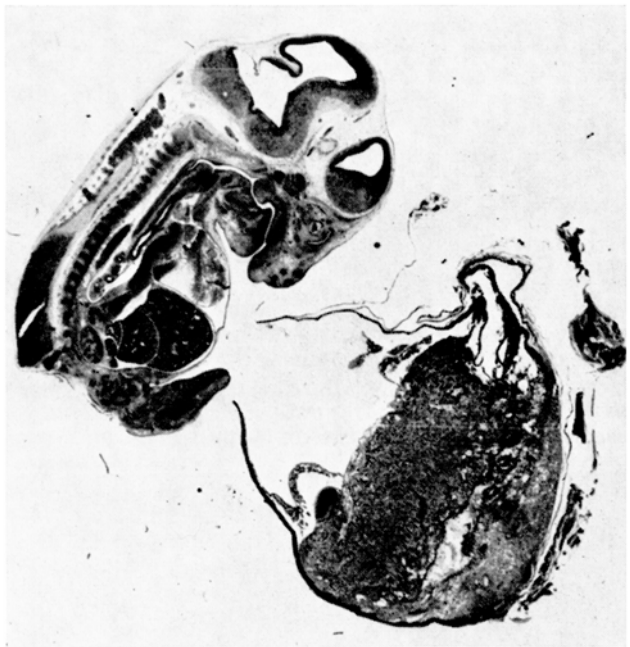
The effects of 5-azacytidine were followed in foetuses aged 14 and 7 days. The drug was administered i.p. at the level of 1.5 mg/kg to pregnant mice on 3 consecutive days. The animals were sacrificed 24 h after the last injection.

In the first instance (14th day of gestation) the number of absorbed foetuses was 20% higher after 5-azacytidine than in controls where 10% of embryos were absorbed. Gross developmental abnormalities were present in the cranial parts and in the livers of treated foetuses. The nervous tissue was largely destroyed and only few pycnotic cells were left. Similarly hepatocytes greatly diminished in number and dividing cells were absent. These changes were present also in the placentae which were considerably smaller than in controls (Figure 1a, b).

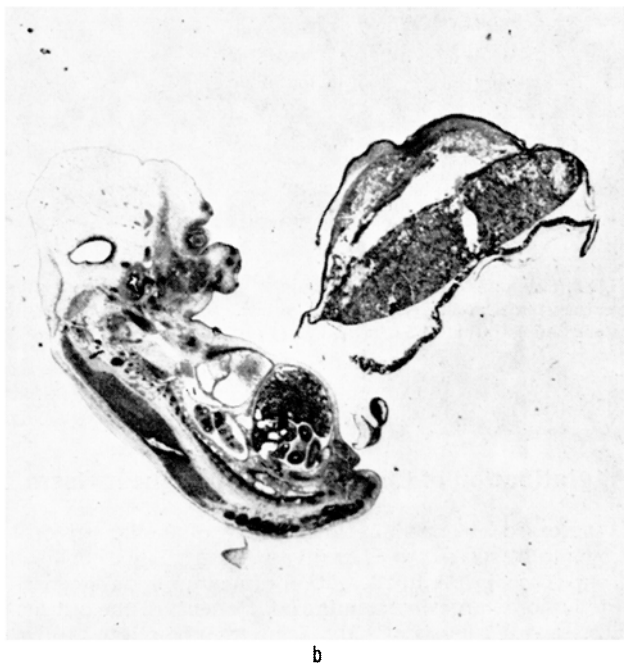
In the second instance (7th day of gestation) foetuses were completely destroyed, and only cells with pale cytoplasma and pycnotic nuclei remained at the site of their nidation. The cells of decidua basalis were in general well preserved; only few cells with vesicular nuclei appeared abnormal. Uterine epithelial and giant cells did not show any aberrations.

Both groups of mice (14th and 7th day of gestation) were injected with thymidine-^{3}H (60 μCi 4 h before killing). In the first group of embryos, the autoradiography did not reveal any silver grains either over livers or central nervous tissue, although in the control foetuses these organs were comparatively heavily labelled (60% of cells were positive with 5 grains over average labelled cell). In the second group label was present only over well preserved cells of decidua basalis and over uterine epithelium including giant cells.

On account of these effects observed in the embryonal nervous tissues after 5-azacytidine, a more detailed investigation was undertaken by administering a single dose of the drug (4 mg/kg) on the 12th day of gestation; thereafter, the pregnant mice were sacrificed at 4, 8, 12 and 24 h intervals and nervous tissue of foetuses was examined histologically. In the controls the region of ventricular columnar epithelium (Figure 2a) was composed of cells with peripheral and central processes, their cytoplasma was rather pale and their nuclei well stained[5–7]. In the ependymal zone mitotic cells at various stages could be seen. It was in this region that prophasic and metaphasic cells with thickened and irregularly arranged chromosomes accumulated 4 h (Figure 2b) after the administration of 5-azacytidine; also pycnotic nuclei were more numerous in comparison with the controls. At 8 h



a



b

Fig. 1. Effect of 5-azacytidine on liver, placenta, and brain of 14-day-old mouse embryos. (a) Control. (b) After administration of 3 doses of 5-azacytidine (1.5 mg/kg/day; 11- to 13-day-old mouse embryos).

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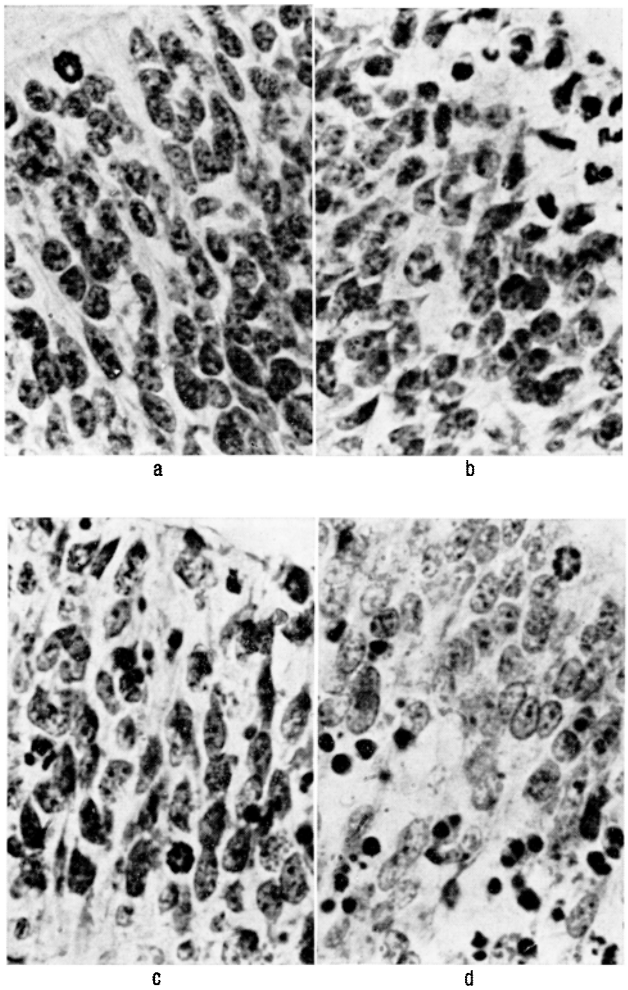

Fig. 2. Columnar epithelium of brain of 12-day-old embryos after treatment with 5-azacytidine. (a) Control. (b, c, d) 4, 8 and 12 h after administration of 5-azacytidine (4 mg/kg).

(Figure 2c) remnants of pycnotic nuclei and mitotic cells with irregular arrangements of chromosomes were still present in the same region. Similar picture, however, could be observed also in the mantle zone. At 12 h (Figure 2d) pycnotic nuclei accumulated in the marginal zone, whereas in the ependymal region normal dividing cells began to appear. At 24 h pycnotic nuclei diminished in size and were present prevalently in the marginal region.

The ability of low doses of 5-azacytidine (1.5–4 mg/kg) to destroy or damage embryonic tissue at doses non-toxic to pregnant mice (LD_{50} 115 mg/kg[8]) led us to investigate its incorporation into embryonal organs. After the i.p. administration of 5-azacytidine-4-^{14}C to pregnant mice (1 μCi/0.25 μmol) the drug was incorporated in an amount of 0.49 mμmol and 0.14 mμmol/mg nucleic acids into foetal liver and central nervous system respectively. It is known that this compound is readily incorporated into different types of nucleic acids[9,10].

Zusammenfassung. 5-Azacytidin wurde trächtigen Mäusen am 4.–6. und 11.–13. Tag verabreicht. Bei der ersten Gruppe zeigte sich eine völlige Resorption der Fetusse, während in der zweiten Gruppe hauptsächlich die Leber und das Nervengewebe geschädigt waren. In der ependymalen Zone werden die in Teilung befindlichen Zellen in der Metaphase gestoppt und unterliegen einer pyknotischen Degeneration.

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Agglutination of Cultured Cells by Wheat Germ Lipase; Effect of Prednisolone

Increased electrophoretic mobility of malignant cells seems to be related to their invasiveness[1]. Since chemical groups susceptible to the action of neuraminidase constitute major charge determining components of the cell surface, elevated levels of sialic acids may be related to the neoplastic properties of such cells. This was shown experimentally by FORRESTER, AMBROSE and STOKER[2] in hamster kidney fibroblasts transformed with the oncogenic polyoma virus. Moreover, malignant cells contain specific sialic acid-rich agglutinogens which are reactive with agglutinins present in preparations from wheat-germ lipase[3,4].

The effect of certain steroid hormones, particularly those of the adrenocorticoid group seems to be associated with changes in the surface properties of treated cells[5,6], possibly involving sialoproteins. Recently CARUBELLI and GRIFFIN[7] have reported that hydrocortisone increases intracellular levels of sialic acid in HeLa cells. It seems that prednisolone stimulates synthesis of sialic acids and does not merely prevent the release of this compound into the culture medium.

In this communication we are presenting evidence to show that agglutinability of cultured cells by wheat germ lipase is increased by exposure to prednisolone during growth. The effect is abolished if prednisolone grown cells are treated with neuraminidase.

HeLa S3 and Henle embryonal intestinal lines were grown for 4 days in glass bottles in the presence of 0.5 μg/ml of prednisolone. The cell monolayers were washed with balanced salt solution and the cells were scraped from glass with perforated cellophane strips. They were then

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