EXPERIMENTAL TUMORS INDUCED IN RATS BY DICHLOROBENZIDINE

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Benzidine and certain other polycyclic diamines are important intermediates in the production of synthetic dyes.

It is known that benzidine is a carcinogenic substance, and that to its action are due numerous cases of cancer of the bladder of workers in the aniline dye industry [1, 3, 5, 6, 9]. There are numerous references in the world literature to the blastomatogenic action of some benzidine derivatives (dianisidine, o-tolidine). Some authors believe that dianisidine may cause tumors of the urinary bladder [1, 10]. Since, however, the same individuals who are exposed professionally to dianisidine are also exposed to benzidine, there is as yet no conclusive evidence of the carcinogenic action of dianisidine. As for o-tolidine, this is not usually considered to be a carcinogen, although in experiments on rats it has been shown to cause tumors of the Zymbal glands [11] in 4.3% of cases.

No data have been published on the carcinogenic properties of dichlorobenzidine (DCB). It is known that DCB can be fairly readily absorbed through the undamaged skin, in particular under conditions of high environmental temperature and humidity [4, 7].

Physicochemical properties. 3,3'-dichlorobenzidine (3,3'-dichloro-4,4'-diaminodiphenyl) is a grey, odorless powder. It is sparingly soluble in water (100 g of water will dissolve 0.07 g of the substance at 15°C).

EXPERIMENTAL METHODS

We used 111 sexually mature white rats, weight 110-130 g, in our experiments. The percentage incidence of spontaneous tumors in these rats is extraordinarily low. Only a few tumors were observed, these being sarcoma of the mesentery, and mammary gland tumors in aged virgin females. As controls, we took a group of 130 rats, which had over a period of 10 months received injections of octadecylamine or methylstearylamine into the subcutaneous areolar tissue. No tumors were found in this group over a period of 20 months of observation.

The animals were on an unrestricted diet. The diet was adequate with respect to animals proteins, carbohydrates, and vitamins. The DCB was supplied by the Voroshilov Scientific Research Institute of Organic Intermediates and Dyestuffs. The product was a paste, consisting of 45.3 parts of the substance to 50 parts of water. It was administered either subcutaneously, as a suspension in glycerol or sunflower seed oil, or per os. The organs of all animals dying or killed were subjected to macro- and microscopic examination.
Feeding of DCB. Fifty rats were taken for these experiments (see Table). From 0.5 to 1 ml of a 4.4% suspension of DCB in sunflower seed oil were added to the daily rations, except on Sundays and holidays, for a period of 12 months.

**EXPERIMENTAL RESULTS**

Acute enlargement of the spleen and liver of one rat was observed, over a period of 10 months from the beginning of the experiments. Microscopic examination of the organs showed the presence of myeloid leucosis.

After 11 months a mammary gland tumor was found in another rat. In all, 12 animals of this group developed tumors. These were variously situated, and 3 of the animals had two different types of tumor (sarcoma of subcutaneous connective tissue with a mammary gland tumor, a thyroid tumor with a hepatoma, a polymorphous cell sarcoma of the wall of a parasitic cyst with a papilloma of the urinary bladder).

Two of the bladder tumors were benign papillomas. One papilloma showed the beginnings of invasive growth into the submucosal layer. The papillomas were found 15 and 16½ months after the start of the experiments.

Tumors of the Zymbal glands (sebaceous glands of the external auditory canal [2]) had an organoid structure, with a preponderance of keratinized squamous tumor cells. The mammary tumors all had the structure of cystoadenocarcinomas; the liver tumors were hepatomas.

**Injections of DCB.** We used 61 rats for these experiments (see Table). From 20 to 120 mg of DCB were injected daily into the subcutaneous connective tissue, for a period of 10-11 months. The injections frequently caused an excited state of the animals, with rigors of short duration. Because of the high mortality encountered, the dosage levels were later reduced, and, beginning from the 6th month of the experiment, the rats were given an injection of 0.5 ml of 8.8% DCB emulsion in glycerol once weekly, i.e., 20 mg per week.

A mammary gland tumor was found in one of the rats after 7 months of the injections. A second tumor, of the Zymbal glands, appeared in this same rat 5½ months after surgical removal of the mammary tumor.

In this series of experiments we found tumors in 18 rats, both at the site of injection and in various...