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EFFECT OF DL-TRYPTOPHAN ON INDUCTION OF TUMORS BY ACRYLIC PLASTIC

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Plastic disks implanted subcutaneously in rats induced sarcomas in 11 of 27 animals (40.7%) surviving until the appearance of the first tumor (12.3 months). No tumors were found at the sites of implantation of powdered plastic. After prolonged administration of DL-tryptophan tumors developed around the disks in six of 19 rats (31.5%) after 13.8 months and around the powder in two of five rats surviving until 18.7 months. In some animals of this group pre-sarcomatous changes were discovered around concentrations of powder.
KEY WORDS: acrylic plastic; tumor induction; tryptophan; fibrosarcoma.

The view is held [3, 4] that the ability of plastic disks to induce tumors can be explained by their ability to deposit endogenous carcinogenic agents, possibly tryptophan metabolites, on themselves. As confirmation of this hypothesis the results of experiments to study implantation of shredded cellophane, previously incubated with urine of intact rats [1], are cited; in 11 of 31 rats which survived 11.5 months sarcomas developed. Under ordinary conditions shredded cellophane did not induce tumors.

Potentiation of the action of several chemical carcinogens by administration of a "tryptophan diet" has been demonstrated by a number of investigations [8, 9]. The effect of tryptophan on the induction of tumors by polymers has not previously been studied.

The object of this investigation was to study the induction of tumors by plastic implants during loading with DL-tryptophan.

EXPERIMENTAL METHOD

Experiments were carried out on 66 noninbred albino rats reared by the writers, weighing 140-160 g, into which the plastic ethacryl (a copolymer of methyl and ethyl esters of methacrylic acid with the methyl ester of acrylic acid) was implanted under ether anesthesia. The powdered plastic, with a particle size of 20-300 μ, was introduced in a dose of 800 mg beneath the skin of the right flank, and a plastic disk of the same weight, measuring $20 \times 20 \times 1$ mm, was introduced subcutaneously into the left flank. The animals of group 1 (17 females and 17 malles) were given 100 mg DL-tryptophan in 0.5 ml sunflower oil per os through a tube five times a week, starting from the day after the operation until the end of their life. The rats of group 2 (16 females and 16 males) received 0.5 ml sunflower oil with their food five times a week. The animals were kept under observation until they died naturally. The material was treated histologically and paraffin sections were stained with hematoxylin-eosin and with picrofuchsin by Van Gieson's method.

EXPERIMENTAL RESULTS

Changes in the connective tissue around the implanted plastic in the rats of group 2 corresponded to those described by other workers [2, 11]. Around the disks during the first days after the operation a picture of

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Fig. 1. Character of connective tissue around implants in animals of group 2. a) Capsule around disk three months after implantation (400 ×); b) thinning and resorption of collagen fibers around particles of powder in 10th month of experiment (230 ×). Hematoxylin-eosin.

Fig. 2. Development of tumors around powder in rats receiving tryptophan; a) Stimulation of proliferation of loose connective tissue cells (230 ×); b) sarcoma induced by powdered plastic; below (arrow) – powder invaded by connective tissue; c) histological structure of same tumor (440 ×). Hematoxyline-eosin.

aseptic inflammation was observed, followed by proliferation of fibroblasts and the formation of a dense collagen capsule around the implant. By the third month of the experiment the proliferative activity of the fibroblasts in all layers of the capsule was reduced, and six months after implantation most of the capsules contained only a very few proliferating cells (Fig. 1a). At the same time, foci of diffuse proliferation of fibroblasts with no signs of atypia, and with very slight polymorphism, appeared in the inner layer next to the implant. Later, polymorphism increased in these areas, atypical cells appeared, and this was followed by the formation of tumor nodules. Tumors always developed from foci of atypical proliferation in the inner layer of the capsule. The first neoplasms appeared 12.3 months after the operation. The number of surviving rats at this time was 27. Altogether in the rats of group 2 tumors were found around the disks in 11 cases (40.7%) after a latent period of 16 months.

By contrast with the disks, cellular activity around the powder remained well marked for a long period of time. Each particle of powder was surrounded by its own thin collagen capsule, with a few young fibroblasts in the inner layer. A delicate network of collagen fibers was observed between these capsules, and among the network there were fibroblasts, varying in their degree of maturity, foreign body giant cells, macrophages, and blood cells. This structure continued until about the 10th month, after which the collagen bundles between the separate capsules and in the capsules themselves became thinner, and in some places they swelled, merged with

one another, and were gradually resorbed (Fig. 1b). Later the particles of powder were surrounded by connective tissue of the ordinary structure with a few cells. No signs of atypical proliferation could be found. No tumors developed in the animals of group 2 at the sites of implantation of powder.

The dynamics of the changes in the connective tissue around the disks in the rats of group 1 was the same as the corresponding pictures described above. Tumors appeared from foci of atypical proliferation in the inner layer in six animals which survived until 13.8 months. By that time 19 rats were still alive. The frequency of appearance of neoplasms was 31.5% and the mean latent period 16.3 months. In their histological structure, tumors accompanying the disks were typical fibroblastic sarcomas of a varied degree of maturity. The dose of tryptophan at the time of appearance of the first tumor was 29.5 g, and the dose corresponding to the mean latent period was 35.6 ± 2.11 g.

Changes around the powder as a rule differed from those observed in the animals of group 2. Increased proliferation of cells, mainly in the loose connective tissue between the encapsulated particles of powder, was found 10-11 months after implantation. Under these circumstances the character of collagen formation changed: the bundles of fibers became coarse and thick and they interwove irregularly (Fig. 2a). Later the relationship between the fibrous and cellular components changed in favor of the latter. The cells became more atypical, their polymorphism increased, and in two animals tumors developed after 18.7 months at the site of implantation of the powder – these were fibroblastic sarcomas (Fig. 2b, c). Five rats survived until this time. The dose of tryptophan at the time of appearance of the first tumor was 40.1 g.

It must be emphasized that the appearance and intensification of atypical proliferation were observed primarily in the connective tissue between the capsules surrounding the particles of powder, and only when the character of the proliferating tissues acquired distinctly neoplastic features could cellular activity be found in the capsules themselves. On the other hand, even in the thicker sarcomatous tissue, particles of powder surrounded by thin collagen capsules could be found, with no signs of proliferation of the cells composing the inner or middle layers.

In some rats kept on a "tryptophan diet," changes of a presarcomatous character were thus found around the powder, and fibrosarcomas were induced in two animals. No such changes were found in the animals of group 2 or in any of the 150 rats in previous investigations [5]. Other workers [1-4] also deny that sarcomas can develop around powder under ordinary conditions. It has recently been shown [7] that with a decrease in the size of the implant the most likely number of pretumor cells, calculated mathematically in a series of concrete cases, and confirmed experimentally, decreases, and for disks measuring $7 \times 15 \times 0.2$ mm implanted into mice it is only 1. These data correlate with the absence of a carcinogenic effect of powdered plastic in the animals of group 2 in the present experiments.

The source of origin of the sarcomas around the powder in the animals receiving tryptophan was the loose connective tissue cells lying outside the collagen capsules surrounding each particle of polymer. The fact that sarcomas developed around the polymer in a form known to be noncarcinogenic (powder) and the absence of any increase in the carcinogenic effect around the disks during tryptophan loading could be an indication of differences in the mechanism of tumor induction in these two cases. Prolonged nonspecific injury with continuous cell proliferation is known to lead to the appearance of a tumor when additional carcinogenic factors of exogenous or endogenous nature are present [10]. Such nonspecific proliferation is observed for a long time around the implanted powder, and this could probably lead to realization of the carcinogenic properties of some tryptophan metabolites. Other evidence in support of this view is given by the fact that the tumors developed, not from cells of the collagen capsules around the particles of powder, as was characteristic of the disks, but from the loose connective tissue, where cell proliferation was most marked. The absence of intensification of the carcinogenic action of the plastic disks during tryptophan loading may be due to a reduction in such proliferation around the implants and its almost total disappearance by the 6th month of the experiment. Under these conditions carcinogenic tryptophan metabolites could not exert their adjuvant effect. Meanwhile, processes of tumor transformation could take place, besides through the intervention of disks, as a result of the same mechanisms, which are still obscure, as in the animals of group 2. Proliferation of cells around implanted disks, as experiments [6] have shown, may be specific in character as nearly as in the first month of the experiment, for during this period cells with pretumor potential have been found. The absence of any increase in the carcinogenic effect under the influence of tryptophan may perhaps also be explained by the neoplastic determination of these changes around the disks during the first weeks of the experiment.

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MECHANISMS OF CHANGES IN WEIGHT OF THE CENTRAL LYMPHOID ORGANS DURING ADENOVIRUS CARCINOGENESIS

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Transplantation of spleen cells of CBA mice at the 25th day of the latent period of carcinogenesis induced by SA7 (C8) virus into newborn syngeneic animals evoked a graft versus host reaction in them. Splenomegaly and a progressive decrease in the weight of the thymus were observed in the recipients. Similar changes in weight of the lymphoid organs were found in animals infected neonatally with oncogenic adenovirus SA7 (C8). The results show that adenovirus carcinogenesis has some manifestations of autoimmune disease.

KEY WORDS: adenovirus; carcinogenesis, autoimmune response; graft versus host reaction; thymus; spleen.

The antigenic profile of tissues undergoing malignant transformation exhibits certain regular features. A common factor in the dynamics of the antigen spectrum of tissues undergoing malignant change is a decrease in the concentration of organ-specific antigens and the accumulation of embryonic antigens associated with tumors [3, 6, 7]. One of the factors which determines the specific character of the antigenic complement of tissues during carcinogenesis is the autoimmune response, frequently observed during carcinogenesis [4, 8]. The writers have shown that in the early stages of carcinogenesis induced by simian adenovirus SA7 (C8) lymphocytes immune to antigens of embryonic fibroblasts appear in the spleens of CAB mice [2].

Considering that immunologic tolerance to certain antigens is due to the functioning of particular populations of co-called T- and B-suppressor cells it is logical to postulate that "injury" to these cells is the cause of the autoimmunity observed in the test system [4, 5, 10]. An expected manifestation of the abolition of the suppressive function may be the increased proliferative activity of a certain population of lymphocytes.

In the present investigation, in order to detect lymphocytes with increased proliferative powers, spleen cells were transplanted from CBA mice in the latent period of carcinogenesis into syngeneic newborn recipients.

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