durations (1–12 h) and allowed to recover in water thereafter. Chromosome squashes are prepared following the conventional Feulgen technique.

Among the experiments with different combinations of variables, the 3 h treatment in 100 ppm and upwards resulted in scattered anaphases and in arrested metaphases with well spread chromosome configurations due to spindle inhibition. Although 25 ppm seems to be the minimal effective concentration when used for 6 h, prolonged treatments in other concentrations resulted in chromosome diminution, thus simulating colchicine. Aqueous recovery of the above materials for 24 h accumulated diplochromosomal metaphases and tetraploid cell populations. The minimal effective concentration of the exotoxin compares well with the known spindle poisons[2,9] at the initial stages of C-mitotic activity, and appears to be slightly more efficient towards the full effect (Table). The similarity of exotoxin with vinblastine and vincristine in this connection is noteworthy.

Since the exotoxin is a known RNA polymerase inhibitor[7], the C-mitotic activity has probably resulted from an interference with the synthesis of proteins in the spindle microtubular systems. A few other abnormalities, like phragmoplast inhibition, chromosome bridges, etc, have also been noticed in higher treatments and are under investigation.

The present study suggests the utility of this bacterial exotoxin as a cytological tool, in view of its spindle-inhibiting properties similar to colchicine. For the same reason, nevertheless, there seems to be a need for caution in the extensive use of commercial preparations of *B. thuringiensis* as a microbial insecticide on crop plants.

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Mast-cell Reaction in Precancerous Mouse Skin: an Immunological Response?

J. F. RILEY

University of Dundee, Department of Pharmacology and Therapeutics, Ninewells Hospital, Dundee DD1 9SY (Scotland), 26 July 1976.

Summary. The promotion phase of carcinogenesis in mouse skin is accompanied by a mast-cell reaction in the upper dermis. Evidence is presented which suggests that this may be an immunological response, whereby distant lymphocytes migrate to the area and, in the presence of young fibroblasts, become transformed into tissue mast cells.

In the course of carcinogenesis in mouse skin a situation develops in the superficial dermis which strongly favours the accumulation of tissue mast cells, the reaction reaching its maximum under a papilloma[1,2]. This curious phenomenon can be induced by promoting agents alone, or, more intensely, by initiation followed by the repeated application of a non-carcinogenic promoter[3,4]. The classical hydrocarbons act in both capacities[5].

The first, and simplest, hypothesis to account for the reaction is to postulate the release of a chemical factor from the overlying, hyperplastic epidermis which acts directly on the dermis[3]. So far, I have failed to identify such a factor. The mast cell in the mouse contains heparin, histamine and 5-hydroxytryptamine, but neither they nor their immediate precursors have proved, on injection, to be capable of eliciting a local development of mast cells. Injections of hyaluronic acid and the chondroitin sulphates, B and C, were likewise ineffective[6].

The absence of mitoses in the new cells, or in the more mature cells already present, suggests a second hypothesis, that the new mast cells stem from distant precursors which only declare themselves when all the conditions for their fulfilment have been met. The first cells, containing a few orthochromatic granules, to appear under the painted epidermis resemble lymphocytes. If this is so, they could either be T-lymphocytes from the thymus, or B-lymphocytes (bursa-equivalent cells) from elsewhere. BURNET[7] has suggested that the mast cell may be an end-cell of the T-lymphocyte. However, mast cells are surprisingly numerous in the untreated skin of so-called 'nude' mice, congenitally lacking a thymus[8]: these cells at least, must stem from non-thymic precursors.

So far as the B-lymphocytes are concerned, CSABA[9] believes that the spleen is the 'mast-cell organ' of the mouse. Yet I have found that splenectomy, prior to painting with DMBA, is without effect upon the emergence of papillomas or upon the mast-cell response[6]. This still leaves a considerable residue of lymphocytes in gut, peritoneum and nodes as possible antecedents for the mast cell, and two lines of evidence suggest that such a transformation may occur.

The first rests on histological findings. Mast cells are rare in encapsulated lymphoid tissue. When, however, non-capsulated lymphoid tissue lies in direct contact with young connective tissue – as in the 'milk spots' of the omentum, the solitary nodules of the gut, or even in lymphoid nodules in the bone marrow – a transformation to mast cells is seen in the boundary zone between the two tissues[2]. Lymphocytes from encapsulated foci must first traverse the lymphatics to reach a suitable site for their full development.

The second line of evidence is immunological. GINSBURG[10,11] has devised an in vitro system in which lymphocytes from a sensitized mouse are cultured on a monolayer of fibroblasts and are there re-introduced to the specific antigen. A massive differentiation into mast cells follows. It will be observed that in both examples,

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cited above, the participation of the fibroblast is essential for success. This symbiotic association of mast cell and fibroblast forms the basis of the 'mast-cell cycle', described elsewhere[2].

If, then, a mast-cell reaction in precancerous mouse skin is to be interpreted as an immunological phenomenon, it would seem necessary to postulate, first, the release of antigenic material from the hyperplastic epidermis and to envisage its contact with distant lymphocytes; second, the re-entry of these lymphocytes into an area which is still yielding specific antigen; and, third, the reunion to occur in the vicinity of young collagen-forming fibroblasts. Viewed in this way, a mast-cell reaction in mouse skin is not an obligatory feature of carcinogenesis: it neither favours nor hinders the development of cancer. Nevertheless, as an 'index of promotion' it may shed light on the mechanism whereby an initiated epidermis gradually acquires the property of invasive growth[3,4].

The Origin of Experimental Brain Tumours: A Sequential Study

P. L. Lantos and D. J. Cox[1]

Department of Neurological Studies and Bland-Sutton Institute of Pathology, Middlesex Hospital Medical School, London, W1P 8AA (England), 20 April 1976.

Summary. A sequential study of rat brains treated transplacentally with the neurotropic carcinogen ethylnitrosourea reveals small foci of cell proliferations from the age of 8 weeks. These lesions consist mainly of undifferentiated cells of the subependymal plate type. They occur in those areas in which gliomas develop and represent the earliest, histologically detectable, changes in the development of brain tumours.

Ten years ago Druckrey et al.[2] reported that a single i.v. injection of *N*-ethyl-*N*-nitrosourea (ENU) into pregnant rats induced tumours and malformations in the offspring. Since then ENU, a simple nitrosamide, has proved to be the ideal carcinogen in the study of experimental neural tumours. A single dose of this compound administered to foetal and neonatal rats induces a high incidence of neoplasms selectively and consistently in the nervous system. The strong carcinogenic action of ENU on the nervous tissue, however, is restricted to the perinatal period: no tumour develops when ENU is administered to pregnant rats before the 12th day of gestation and the susceptibility of the nervous system to ENU also decreases with increasing age[3,4].

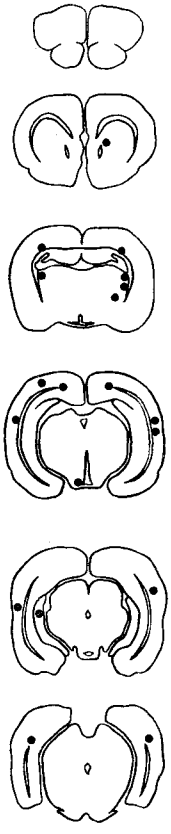

Fig. 1. Diagrammatic representation of the distribution of lesions described in the text.

Histologically the tumours are gliomas of the central nervous system and schwannomas of the cranial and peripheral nerves, although neuroblastomas have also been reported[5]. Multiple tumours are frequent: neoplasms of macroscopic size, microtumours and early neoplastic proliferations are all present in the same animal[6]. Cerebral tumours are not distributed haphazardly but occur in certain preferential sites: the periventricular area and the subcortical white matter of the cerebral hemispheres are most frequently involved.

Since most studies have been carried out on large, fully developed tumours which eventually killed the animals, after an average of 245 days, very little is known about the early stages of tumour growth. A sequential analysis of ENU-induced schwannomas of the trigeminal nerves was recently published[7], but similar studies are lacking on the earliest stages of the development of cerebral gliomas. The purpose of the present communication is to describe the development of such ENU-induced gliomas.

Material and methods. A single i.p. injection of 40 mg of ENU per kg of body weight was injected into pregnant BD-IX rats on the 15th day of gestation. The ENU was dissolved in a 3 m*M* citrate buffer containing 0.9% (w/v) sodium chloride, pH adjusted to 6.0 at 20 °C. Con-

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