Mast Cells, Co-Carcinogenesis and Anti-Carcinogenesis in the Skin of Mice

It is now widely accepted that chemical carcinogenesis in the skin of the mouse is a 2-stage process, initiation followed by promotion (co-carcinogenesis)[1,2]. Initiation seems to imply some form of genetic change: it is more easily achieved in mitotically active skin than in skin in which the synthesis of DNA has been temporarily suppressed[3,4]. The altered cell may then lie dormant throughout the life-span of the individual, unless forced to declare itself through the persistent action of a promoter. The carcinogenic hydrocarbons are 'complete' carcinogens, initiators and promoters. However, by applying the hydrocarbon once only and in low dosage, its action can be limited to that of an initiator. Hitherto, the most potent promoter has been croton oil[5], from which HECKER[6] and his group have recently isolated a highly active principle named, for convenience, 'Compound A_1'. Certain other substances have an opposite effect on carcinogenesis: such anti-carcinogens are mustard gas[7], bromo-benzene[8] and ethyl phenyl propiolate[9,10] (Figure 1). Armed with pure initiators, pure promoters and their respective antagonists, we are now in a position to devise model experiments on mouse skin in the hope of identifying morphological changes which are of significance[11]. The present report is particularly concerned with the participation of the tissue mast cell in carcinogenesis when minimal doses of the above agents are employed.

From the early days of tar-painting it has been realized that both epidermis and dermis are involved in the response of mouse skin to the surface application of a carcinogen[12]. Epidermal hyperplasia, with damage to hair follicles and sebaceous glands, is accompanied by fading of the basement membrane and a teazing out and loss of refractility of the subepidermal collagen[13]. Concomitantly there gradually develops a mast-cell reaction within the dermis of the treated area[14,15]. Normally the mast cells in mouse skin are disposed in 2 layers, 1 in the upper dermis, and a deeper layer in relation to the muscular 'panniculus carnosus'. These are the typical mast cells of Ehrlich, filled with basophilic, metachromatic granules. The carcinogenic mast-cell reaction begins even more superficially, with the appearance of small poorly granulated, orthochromatic cells immediately under the hyperplastic epidermis. These cells increase in number, size and granularity to reach a maximum at the base of a papilloma (Figure 2). With the final transformation to cancer the mast-cell reaction is over-run. 2 questions thus arise. Is a mast-cell reaction a significant feature of carcinogenesis in mouse skin? If so, what does it signify?

The mast-cell reaction and promotion. Present evidence suggests that the mast cell is involved in the promotion phase of carcinogenesis[11]. Thus, some years ago, Professor KAI SETÄLÄ of Helsinki permitted me to stain for mast cells adequately fixed histological material from mice which had been painted repeatedly with various carcinogens and co-carcinogens. On the basis of the mast-cell reaction the series could be arranged in descending order of effectiveness: hydrocarbons, croton oil, Tween-60, Tween-20, oleic acid and Span-60; that is, according to their potency as promoters. The mast-cell reaction was always greater in promoted skin which had first been initiated. A single small initiating dose of hydrocarbon, or urethane, alone left no detectable histological change in the skin.

Now, through the kindness of Professor HECKER in supplying me with a sample of Compound A_1 (the active principle of croton oil), I have been able to re-examine the sequence of events in mouse skin during carcinogenesis. A minute dose of 7,12-dimethylbenz (α) anthracene (0.005% in acetone) was applied once, as an initiator, by means of a single brush-stroke over the centre of the back. The controls received acetone only. 1 week later

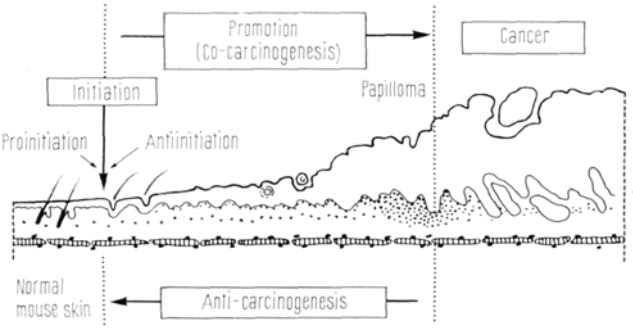

Fig. 1. A composite representation of mouse skin undergoing carcinogenesis (left to right). The black dots are the mast cells. Note the progressive development of a new mast-cell reaction immediately below the hyperplastic epidermis, reaching a maximum under a papilloma.

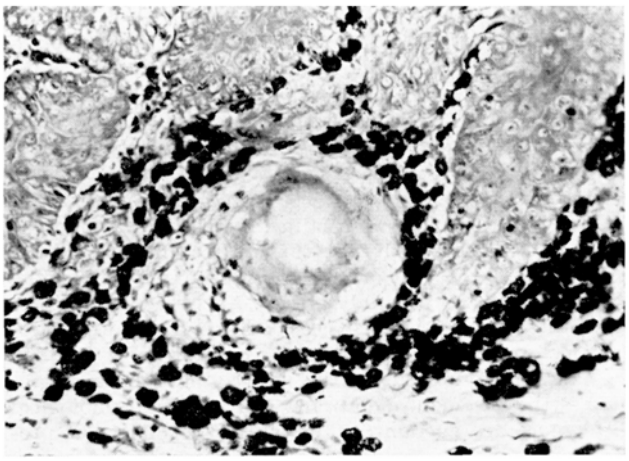

Fig. 2. Mast cells (dark objects) at the base of a papilloma. Their intimate association with the hyperplastic, pre-cancerous epithelium suggests a functional relationship. In the centre of the field is a remnant of a hair follicle. Toluidine blue. × 215.

[1] J. C. MOTTRAM, J. Path. Bact. *56*, 181 (1944).
[2] I. BERENBLUM, N. S. HAROUR and N. TRAININ, Br. J. Cancer *12*, 402 (1958).
[3] H. SHINOZUKA and A. C. RITCHIE, Int. J. Cancer *2*, 77 (1967).
[4] R. SÜSS, Nature *217*, 752 (1968).
[5] I. BERENBLUM, Br. J. Cancer *1*, 379 (1947).
[6] E. HECKER and H. U. SCHAIRER, Z. Krebsforsch. *70*, 1 (1967); Nature *217*, 563 (1968).
[7] I. BERENBLUM, J. Path. Bact. *32*, 425 (1929).
[8] H. CRABTREE, Cancer Res. *4*, 688 (1944).
[9] U. SAFFIOTTI and P. SHUBIK, Conf. Biol. Cutaneous Cancer (Nat. Cancer Inst. Monograph No. 10, 1963), p. 489.
[10] J. F. RILEY and D. M. SHEPHERD, Experientia *21*, 498 (1965).
[11] J. F. RILEY, Lancet *2*, 1457 (1966).
[12] W. H. WOGLOM, Arch. Path. *2*, 533 (1926).
[13] J. W. ORR, J. Path. Bact. *46*, 495 (1938).
[14] W. CRAMER and W. L. SIMPSON, Cancer Res. *4*, 601 (1944).
[15] J. F. RILEY, *The Mast Cells* (Edinburgh 1959).

Hecker's Compound A_1 (1.5 mg/100 ml acetone) was similarly applied to both groups 3 times a week for the next 20 weeks, by which time the initiated-promoted group were developing multiple papillomas. With the above dosage, there was no ulceration at any time, merely a slight epilation over the painted area. Mice were killed at intervals of 3–4 weeks and the entire treated area was fixed immediately in Baker's calcium-formol, blocked in paraffin, cut at 8 μ and stained with toluidine blue at pH 2.0 for mast cells.

The results fully support the hypothesis, previously expressed[11,15], that a mast-cell reaction in some way reflects the changes occurring in the upper dermis during the promotion phase of carcinogenesis: the connective tissue core of the first small papilloma to appear at 12 weeks was filled with mast cells. However, a further control was made by repeating the above experiment after adding to the Compound A_1 0.0005% sodium selenide, an antioxidant which, as SHAMBERGER and RUDOLF[16] find, nullifies completely the promoting effect of crude croton oil. No mast-cell reaction now occurred in either initiated or non-initiated skin and no tumours emerged.

Mast cells and anti-carcinogens. For a long time it was believed that epidermal cancer can be caused by 'chronic irritation' alone. Now it seems more probable that prolonged mechanical irritation is merely a contributory factor in carcinogenesis: a co-carcinogen. The effects of chemical irritants are more complex. Many years ago BERENBLUM, investigating various forms of chronic irritation on the tarred skin of mice, found, unexpectedly, that certain chemical 'irritants' are, in fact, anti-carcinogens. The first to be discovered was mustard gas[7]. Subsequently, CRABTREE[8] showed that many halogenated compounds behave in this way. Recently, a yet more powerful anti-carcinogen has been found in ethyl phenyl propiolate[9,10].

Since carcinogenic hydrocarbons and promoters stimulate an epidermal hyperplasia in the intact skin, it might have been anticipated that the anti-carcinogens would have an opposite effect, inhibiting the growth of the epidermis. This is certainly not so. Anti-carcinogens provoke an epidermal hyperplasia as do carcinogenic hydrocarbons and the non-carcinogenic promoters. It is when we examine conditions in the underlying dermis, beginning at the basement membrane, that clear morphological differences are encountered between the effects of pro- and anti-carcinogenic agents on mouse skin. Prolonged treatment with the anti-carcinogen, ethyl phenyl propiolate, provokes an epidermal hyperplasia, with associated epilation and destruction of sebaceous glands, as does a typical carcinogenic hydrocarbon. But instead of subepithelial fibrillary collagen and a progressive mast-cell reaction, there develops instead a distinct broadening and stabilization of the basement membrane zone. The hyperplastic epidermis now rests firmly on a base of poorly refractile connective tissue: this is sometimes so thick as to be visible on gross examination of the tissue block. Such mast-cell reaction as develops lies deep to this.

Thus, by refining the experimental conditions, step by step, from painting the skin with crude coal tar to the use of pure initiators, promoters and anti-carcinogens, at low dosage, we arrive at a point at which at least 1 morphological feature differentiates pre-cancerous (or co-carcinogenic) skin from hyperplastic normal skin (or skin treated with an anti-carcinogen). This is the basement membrane zone, consisting of the basement membrane itself and the adjacent thin band of refractile collagenous dermis. An index of the trend towards cancer is the gradual and progressive development of a mast-cell reaction within this zone. The fact of such a reaction is now established: its significance remains to be determined[17].

Zusammenfassung. Wenn auf der Haut der Maus eine Karzinogenese mit minimalen Dosen eines reinen Initiators und eines reinen Promoters (Co-karzinogen) induziert wird, tritt eine Mastzellreaktion auf. Diese Reaktion erreicht ein Maximum unter den Papillomen. Sie kann durch Applikation eines Antikarzinogens verhindert werden.

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[16] R. J. SHAMBERGER and G. RUDOLPH, Experientia *22*, 116 (1966).

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Role of Symbiotes in Tanning of Termite Cuticle

In a recent study on the role of symbiotes in termites, *Reticulitermes assamensis* Gardner, it was found that the flagellate symbiotes in the hind-gut of workers, as in *Zootermposis angusticollis*[1], die shortly before each ecdysis and the recently moulted and defaunated workers regain their infections by the solicitation of proctodael 'food', which contains active protozoa, from their non-moulting associates. But, when the freshly moulted defaunated workers were prevented from reinfection, they displayed abnormal symptoms. They became less active and their abdomens were seen to be smaller and slightly flattened. The length of the time required for abnormalities to appear depends on the kind of food fed after ecdysis, the more decayed the wood, the longer it is before any abnormalities appear. In 2 or 3 days after this first symptom was noticed the abdomen became still more flattened. Death occurred, in some instances in less than 15 days, and in a few cases after 20 days, the longest being 30 days. It was striking to notice that the defaunated workers were lighter in colour than the re-infected ones which became deep amber coloured 5 days after ecdysis. Similarly when the workers were artificially defaunated by exposing to 45 lb of O_2 for 1.5 h, they moulted, but they failed to become deep amber coloured as the normal ones. This recalls the report of SCHNEIDER[2] in *Sitophilus*

[1] W. L. NUTTING, Biol. Bull. mar. biol. Lab., Woods Hole *110*, 83 (1956).

[2] H. SCHNEIDER, Naturwissenschaften *41*, 175 (1954).