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## Malignant Transformation and Maturation in Non-Dividing Cells During Polymer Tumorigenesis.* (31822)

K. GERHARD BRAND, LANCE C. BUOEN, AND INGE BRAND
*Department of Microbiology, University of Minnesota Medical School, Minneapolis*

Many theories of carcinogenesis have a common denominator: the concept that development towards malignancy can be regarded as an evolutionary process which stretches over a chain of premalignant cell generations gradually selecting for more autonomous clones. A contradiction is provided by certain viral tumors. Here, infection of a cell with the virus virtually coincides with complete malignant transformation. However, so far this mechanism is being considered exceptional rather than generally compatible with other forms of carcinogenesis(1).

Investigations have been carried out in this laboratory on tumorigenesis following subcutaneous implantation of plastic film pieces into mice. Results already published(2) have changed previous views regarding the site of carcinogenic development in that the premalignant cells were exclusively found to be firmly attached to the plastic film inserts until shortly before tumors become palpable. Experiments in this new direction have yielded data which strongly suggest that the carcinogenic events take place in a non-dividing cell population remaining stationary throughout the premalignant phase.

*Experiments and results.* The design of the experiment was similar to that described previously(2). Inbred mice (CBA-$T_6$ carrying the $T_6$-marker chromosomes) at the age of 1½ to 2 months received in both flanks subcutaneous inserts of vinyl chloride acetate-coverslips ("dispo-slips"), 15 × 22 mm in size. Inserts and tissue capsules (which surrounded the inserts as a result of a foreign body reaction) were cut in thirds after time intervals of from 2 weeks to 16 months. One portion was left in the original animal. A second portion was used for cultural, karyological, and/or histological examinations. The plastic and capsule pieces of the third portion were carefully separated and then individually transplanted into CBA-H recipient mice (which are syngeneic with CBA-$T_6$ mice but do not carry $T_6$-chromosomes). Tumors, with specific $T_6$-chromosomes, appeared simultaneously up to 8 months later in the original and the corresponding recipient animals if transplantation was carried out 6 or more months after initial insertion of the plastic films. If the latent period (*i.e.,* from time of transplantation until tumor appearance) was observed to last longer than 4 weeks, the tumors developed from transplanted film pieces only, not from capsule tissue. These results were in agreement with those described before(2). Evidently, the carcinogenic events take place among cells residing in multiple foci on the plastic film but not in the surrounding tissue as was claimed in the earlier literature (recently reviewed by Bischoff(3)).

As outlined in the foregoing, plastic film fragments were put aside from each single transfer experiment. Accordingly, these fragments were known to carry cell populations with exactly determined degrees of premalignant maturation in terms of time elapsing until tumor appearance. The film fragments were treated in various ways. They were stained immediately after removal from the original carrier animal with Pappenheim's combined May-Gruenwald-Giemsa procedure or with a combined Tetrazolium-Feulgen procedure developed by us for this purpose(4).

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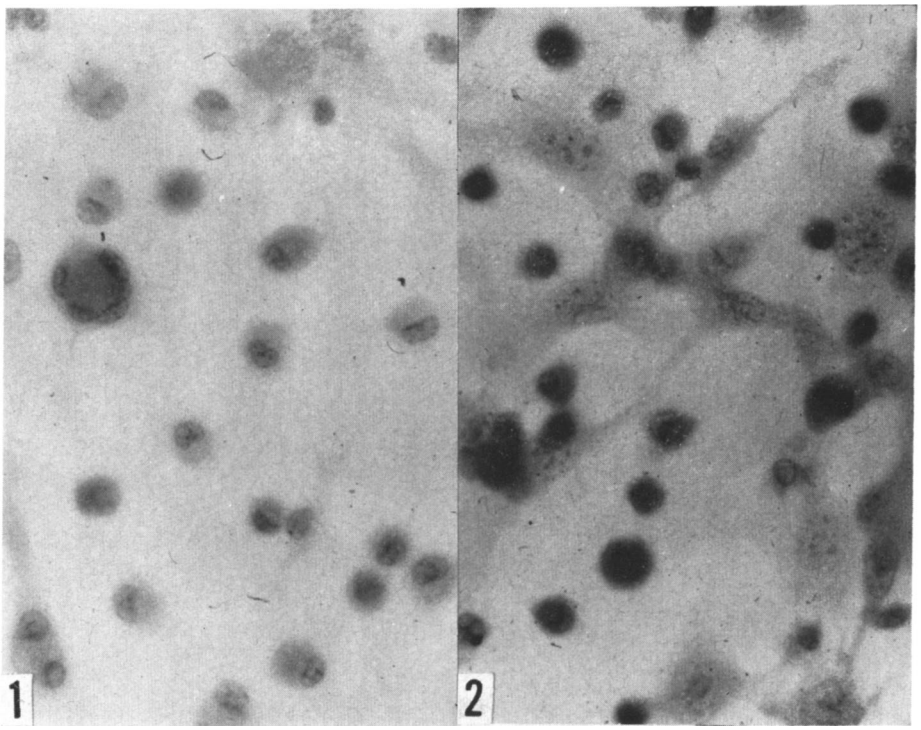


FIG. 1. Cell population on plastic film piece 5 months after subcutaneous insertion into a CBA-$T_6$ mouse. Stained with the combined Tetrazolium-Feulgen procedure(4). Normal bright field, low power objective. Total magnification × 800.

FIG. 2. The same cell population as in Fig. 1 after 3 days *in vitro* culture. Stain and microscopy as in Fig. 1.

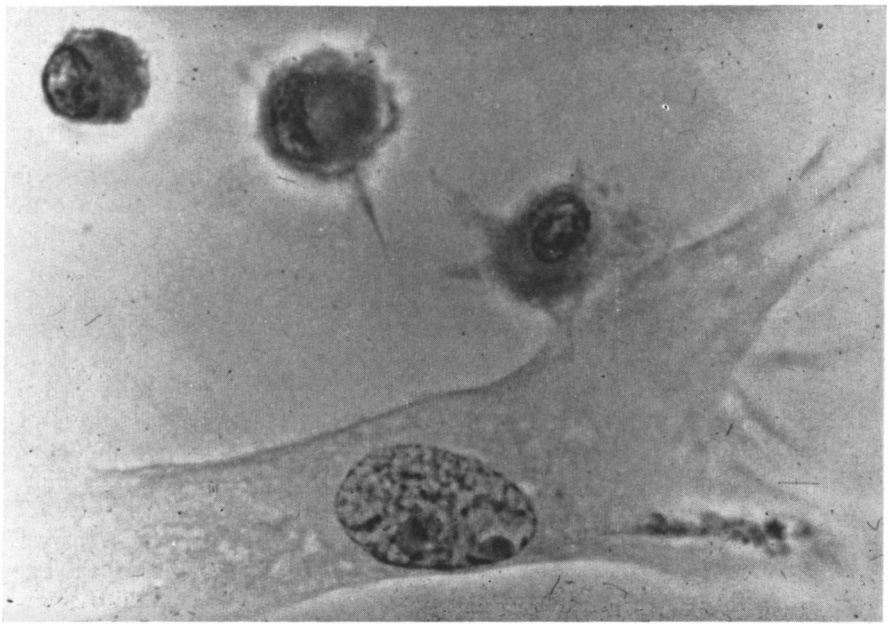

FIG. 3. Same as in Fig. 2. Oil immersion and phase contrast after combined Tetrazolium-Feulgen reaction. Total magnification × 2000.

Other film fragments were used to culture the cells for 3 days according to standard tissue culture methods in McCoy's medium 5a supplemented with 20% newborn calf serum. The cultured cells were then also stained or they were studied karyologically after application of colcemide and a standard air drying procedure to obtain chromosomal spreads.

Microscopic comparison of stained non-cultured film fragments from various stages of premalignant maturation did not show any significant shift in number of cells per area of film. Since premalignant cells had been proven to be present only on these plastic film pieces and not in the surrounding tissue, it followed that the total number of premalignant cells seemed to remain stationary while the cells actually gained malignant maturity. This conclusion was supported by unsuccessful attempts to detect mitotic figures among the film-attached cell population. Negative results were also obtained when mice which had been carrying plastic films for various lengths of time were treated with colcemide 24 hours prior to film removal. As shown in Fig. 1 and 3, most of the cells residing on the plastic film belong to the monocytic macrophage type. Multinucleated cells are often found in great numbers, whereas typical large fibroblasts are scarce and scattered. During *in vitro* culture of coverslips for 3 days, single and apparently normal fibroblasts began to divide. Like a network they finally overgrew the main cell population which either seemed to die off or was morphologically unchanged and, therefore, clearly discernible (Fig. 2, 3). Chromosomal counts on those cells dividing never matched the counts obtained later on tumor stem lines.

Other experiments were designed to assess cellular turnover on plastic film implants more directly. India ink was dried onto round plastic and glass coverslips of equal size (18 mm in diameter) before insertion into mice. (Glass coverslips were used as a "negative" control since the tumor incidence resulting from implanting glass had been found to be almost negligible during the time period observed.) Coverslips and surrounding capsule tissues were removed for direct staining or histological sectioning after various lengths of time. The presence and density of carbon particles extracellularly or intracellularly on the coverslips and in the tissues, as well as the type of cells involved in phagocytic activity, were determined. It was observed that glass coverslips did not hold the carbon particles for very long. After a few weeks the coverslips were found to be "clean" and also the surrounding tissues contained hardly a trace. The carbon particles were presumably transported away into the regional lymph tissue. The plastic material, in contrast, retained a large portion of the carbon particles all through the premalignant period, particularly in the center parts of the round coverslips. Carbon particles were also found in the capsule tissues in rather close proximity to the insert, although the phagocytic cells appeared to be morphologically somewhat different from the main cell type residing on the coverslip.

The finding of carbon particles being retained less firmly in the periphery of round plastic coverslips points to another factor of importance: tissue irritation and sterile inflammation. Throughout these investigations it was observed that the likelihood of a tumor to develop diminished as tissue irritation increased, which manifested itself by leucocytic infiltration and/or fibroblastic proliferation. Even under ideal circumstances this occurred at least around the edges of the inserted coverslips. Accordingly, tumors developed most often from the center of inserts where cellular turnover (and clearance of carbon particles) was minimal in contrast to the periphery.

*Discussion.* From the results accumulated so far in this laboratory the following sequence of events seems to emerge. The main cell population settling down on the plastic film insert enters into a stationary phase which last for many months. Gradually cells transform towards malignancy, apparently without ever dividing. It even seems that prolonged and undisturbed quiescence is one of the conditioning factors. The transformation occurs in many cells at the same time and at the same pace so that fragments of the same insert will produce tumors in different recipient animals after equal time intervals. In less

than 4 weeks before tumors become palpable the (pre)malignant cells detach from the plastic film and can now also be demonstrated in the surrounding tissue.

It is proposed that complete malignant transformation of cells in polymer tumorigenesis occurs during a state of prolonged non-division, not precluding consecutive mutations and selection of the fittest stem line. Data obtained by Shelton *et al*(5) and Evans *et al*(6) during *in vitro* experiments on malignant transformation of mouse tissue are in support of this view. It is suggested that other types of tumors be re-examined for the possible general validity of such a hypothesis. "Dormant" non-dividing cells should be taken into consideration as the actual originators of malignancy even if the premalignant process might be otherwise characterized by initially benign proliferation.

*Summary.* It was confirmed that, during polymer solid surface carcinogenesis, premalignant cells reside on the plastic insert as early as eight months before tumor maturation. No premalignant cells were demonstrated in the surrounding tissue except for the very late stage of less than four weeks. During the premalignant period no mitotic activity or cellular turnover was observed among the cell population that presumably included the premalignant clone. Clearance of carbon particles was minimal. It was concluded that at least in this instance the entire process of premalignant development and maturation seems to take place in non-dividing cells.

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## Effect of Temperature and Drug Therapy on Anthrax Intoxication. (31823)

FREDERICK KLEIN, RALPH E. LINCOLN, BILL G. MAHLANDT, JAMES P. DODDS, AND JERRY S. WALKER (Introduced by Leonard A. Mika)
*Department of the Army, Fort Detrick, Frederick, Md.*

Stress of the test animal with low ambient temperature changes the toxicity of bacterial toxins(1,2). Cold temperature has also been known to influence the action of certain drugs in warm blooded animals(3,4,5,6,7). Recently, Stahnke(2) interacted these two independent variables (temperature and drug) with rattlesnake venom and scorpion venom and observed that both venoms increased in toxicity in the rat under cold stress. Rats receiving the drug epinephrine reacted similarly. In a similar study with *S. marcescens* endotoxin, mice were made more susceptible to the lethal effects of the toxin following cold exposure. Previte and Berry(1) showed that cortical hormones effectively counteracted the toxicity of the toxin at both room and low temperatures. Both studies attributed these apparent changes in toxicity to the physiological effects of stress rather than the temperature *per se*.

In this paper we report initial experiments which suggest that temperature and certain drugs alter the susceptibility of the rat to anthrax toxin. The results indicate that reactions were the opposite to other reported bacterial toxins and snake venoms and suggest alterations other than physiological stress.

The studies were based on the proven homogeneous susceptibility of the Fischer 344 rat to anthrax toxin(8) and the precise assay procedure for anthrax toxin developed utilizing this animal(9). Anthrax toxin was pre-