

Sarcomas at Sites of Subcutaneously Implanted Bakelite Disks in Rats

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INTRODUCTION

On November 6, 1936, a bakelite¹ disk, which had been coated with a very thin layer of 1, 2, 5, 6-dibenzanthracene by a vacuum distillation process, was implanted subcutaneously into the right side of an albino stock rat. An uncoated bakelite disk was implanted similarly into another albino rat, of undetermined origin, as a control. The test disk had been prepared by Dr. Egon Lorenz, of this Institute, for a study of the biological action of thin layers of carcinogenic hydrocarbons. Twenty days later the test disk was removed from the animal in order to observe the effect on the layer of hydrocarbon. No visible change was evident. The disk was not reimplanted. This rat died without tumor 11 months later, of cerebral abscess.

The control rat bearing the uncoated bakelite disk was killed 23 months after the disk had been implanted. A tumor surrounding the bakelite disk measured 21 x 33 x 49 mm. Dr. Hugh Grady, of this Institute, examined the tumor and reported that it was—

an atypical fibrosarcoma composed of small bundles or nests of cells which varied from spindle to bizarre round or pear-shaped elements with large hyperchromatic nuclei. There was a heavy network of collagen fibers closely attached to many of the tumor cells, and small numbers of irregular, poorly formed blood vessels were seen. In phosphotungstic acid haematoxylin prepara-

tions fibroglia fibrils were identified in many tumor cells. Mitotic figures were numerous.

Transplants of this tumor into two Wistar strain rats did not grow.

Several investigators (1-3) have tested formaldehyde under varying conditions. None reported tumors following its use. No record was found to show that sodium sulfite, phenol, paraform, or orthocresol had been tested for tumor formation. Paracresol, which is chemically somewhat similar to orthocresol, was tested by Reimann (4), who made repeated applications to the skins of both rats and mice and observed no tumors after 6 weeks. Hexamethylenetetramine has been injected repeatedly into male mice by Dr. Shear and Dr. Stewart, of this Institute.² No tumors were observed in 5½ months. Bogen and Loomis (5) observed one benign tumor after having painted the skins of white mice for more than a year with caustic soda. Twort and Fulton (6) made repeated applications of lactic acid to the skins of mice and observed no tumors in 25 weeks. Petroff and Krotkina (7) used nine rats into which they made injections into two sites. Into one site was implanted embryonic tissues, and into the other, embryonic tissues plus lactic acid. Tumors occurred at the sites of the embryonic pulp, and in one rat a tumor arose at the site of the embryonic tissues plus lactic acid. Nakahara and Mori (8) fed glycerin to rats for more than 300 days. They observed neither tumors nor any other significant pathological condition. Hence,

¹ Bakelite is formed by the chemical interaction between phenol and formaldehyde. In some products, a crude phenol, orthocresol, is used, and in others, paraform, a polymer of formaldehyde, is employed. As catalysts, the following substances have been used: caustic soda, sodium sulfite, hexamethylenetetramine, lactic acid, and glycerin.

² Shear, M. J., and Stewart, H. L.: Unpublished data.

no very conclusive evidence of carcinogenic activity was obtained by any of the ingredients of bakelite, by the methods reported.

EXPERIMENTAL PROCEDURE

EXPERIMENT 1

On October 4, 1938, squares of bakelite 10 x 10 x 1½ mm. were implanted subcutaneously into the right sides of 10 male strain dba mice 2½ months old. The last mouse died at the age of 18 months. No tumors were observed in any of the mice. There was no apparent change on the surface of the bakelite squares.

EXPERIMENT 2

On December 16, 1938, a bakelite disk was implanted subcutaneously into the right side of each of six male and six female Wistar strain rats 6 weeks old. The bakelite disks were 1½ mm. thick and 18 mm. in diameter. The surface of each disk had been removed with a new steel file after which the disks were scrubbed with a brush, soap, and hot water, rinsed in tap water, boiled in distilled water, and implanted.

Several of the rats died early of pneumonia. The first palpable mass at the



FIGURE 1.—Gross appearance of tumor which developed around a bakelite disk. The other tumors had a similar appearance.



FIGURE 2.—Fibrosarcoma showing bundles of spindle-shaped cells. Phosphotungstic acid haematoxylin stain. $\times 350$.

site of a bakelite disk was observed 18 months after the disks had been implanted. Eight rats, five males and three females, were alive on that date. Of these eight rats, three developed tumors at the sites of the bakelite disks.

The pathological diagnoses of these were also made by Dr. Grady. He found that—all three tumors were fibrosarcomas and were composed of interweaving bands of long, spindle-shaped cells with tapering processes and darkly staining nuclei. Moderate amounts of collagen were present in two instances, while in the third tumor large collagenous bundles were observed. Fibroglia fibrils were easily identified in all three tumors and mitotic figures were present.

No attempt was made to transplant the tumors (figs. 1 and 2). In three tumors a brownish-orange-colored fluid was in contact with the bakelite disk. In one instance, the disk was inclosed in a cyst containing this fluid. Histopathological examination of this fluid by Dr. Grady

revealed large numbers of mononuclear cells. A spectrographic examination of the fluid by Dr. Herbert Kahler, of this Institute, revealed only components of the hemoglobin spectrum.

DISCUSSION

Disks were implanted into 13 rats. Nine of the rats lived to be 20 months of age, or older; tumors developed in 4, 3 males and 1 female. Bakelite squares were implanted into 10 mice. Six of the mice lived to be 18 months old and had had the bakelite constantly in contact with their subcutaneous tissues for 15 months. This period of time was probably as great a fractional part of the life span as that experienced by the rats, yet tumors developed in the rats but not in the mice. Although the number of test animals was small, the findings suggest that the rats were more susceptible to tumor formation by this agent. What factors underlie this difference are not known; both were fed the same diet, dog chow, except that the rats were occasionally given cod-liver oil because they showed evidence of vitamin A deficiency. This dietary deficiency was not studied in relation to tumor formation.

A tumor, a mammary adenofibroma, developed at the base of the tail of the last surviving rat (female) which lived to be

26 months old. It probably was a spontaneous tumor which arose uninfluenced by the presence of the implanted disk. No tumor was found at the site of the bakelite disk.

It required many months for the tumors to develop around the disks, but they grew rapidly after their initial appearance. There appeared to be very little, if any, erosion of the bakelite disks.

The mode of action of the biological processes involved in the formation of tumors by bakelite is as ambiguous as the *modus operandi* of other carcinogenic agents. Although tumors occurred in the rats and not in the mice, chronic irritation cannot be entirely excluded as an adjuvant factor, for the subcutaneous tissues of the rat may be more favorable to tumor formation than the subcutaneous tissues of the strain of mice employed.

The fact that all of the mice and most of the rats bore the disks continuously in contact with their subcutaneous tissues for most of their entire lifetimes without developing tumors suggests a very low hazard of carcinogenicity.

SUMMARY

Bakelite disks were implanted subcutaneously into 13 rats. Of 9 rats which survived longer than 20 months, 4 developed sarcomas at the site of the disk.

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