Effect of Repeated Surgical Trauma on Chemical Carcinogenesis*

BERNARD GOTTFRIED, NORMAN MOLOMUT, AND JOSEPH PATTI
(Waldemar Medical Research Foundation, Port Washington, N.Y.)

SUMMARY
C57BL/6 mice given subcutaneous injections of a tumor-producing dose of 3,4,9,10-dibenzpyrene were subjected to either skin wounding or laparotomy. Tumors were induced earlier and progressed more rapidly in the mice subjected to either of the surgical procedures. The mice subjected to laparotomy exhibited the greatest effect of tumorigenesis compared with that in control and skin-wounded mice. The data indicate that surgical trauma acts as a co-carcinogen, augmenting the carcinogenic effect of 3,4,9,10-dibenzpyrene.

We are investigating the influence of surgical trauma on the development and progression of experimental tumors. Our initial experiments were conducted with transplanted tumors in mice (3,4), in which we found that repeated skin wounding shortened the latent period after tumor graft and accelerated the rate of tumor growth. The present experiment was designed to approximate more closely natural tumor occurrence by the use of a chemical carcinogen to induce neoplasia.

Deelman (1) studied the effect of wound trauma at the site of application of the carcinogen, creating a local inflammation and injury added to that caused by the carcinogen. He reported an increase in the number of tumors induced by the carcinogen and wounding. Werder, Hardin, and Garth (7) reported an increase in the number of mice with methylcholanthrene-induced tumors following a single surgical skin incision, sutured to heal by primary intent. In our studies with tumor grafts, we found that repeated surgical trauma was needed to effect a demonstrable increase in tumor growth.

MATERIALS AND METHODS
C57BL/6 mice originally obtained from the National Institutes of Health and inbred in our colony since 1956 were used. In total, 259 mice, approximately one-third females, 10–15 weeks old,

* This investigation was supported in part by grants from the National Cancer Institute, National Institutes of Health, United States Public Health Service and the American Cancer Society.

Received for publication December 1, 1960.
but not including the dorsal muscles. Wounds were allowed to heal by secondary intent (by adhesion of granulating surfaces) and surgically reopened 3 times weekly until sacrifice after the 17th week. Surgical asepsis and skin disinfection with 1:5000 hyamine in 70 per cent isopropanol were employed in all woundings. No special procedures were employed or required to maintain noninfected wounds.

Group 3: Carcinogen and laparotomy.—86 C57BL/6 mice were given injections of 3,4,9,10-dibenzpyrene as described. In total, seven mice were sacrificed between the 5th and 6th weeks for histologic evaluation of the carcinogen injection site. The remaining 79 mice were subjected to laparotomy every 2 weeks beginning with the 6th week after the carcinogen injection. Seven mice with the histologic evidence from the sacrificed animals. In addition, the weekly size increment and growth pattern of the tumor was a further index of time of tumor initiation. The presence of all tumors was confirmed by microscopic examination of sections at death or sacrifice.

RESULTS

The data from this study show that surgical trauma exerted a marked effect on the latent period of DBP tumorigenesis. Table 1 summarizes the tumor incidence in each group as determined by weekly palpation. There was a markedly greater incidence of initiation of tumor in both surgically treated groups of mice as compared with the controls.

The percentage of mice with tumors appearing died due to anesthesia and are not included in the final data. A total of 72 mice comprised this group.

Laparotomies were conducted under nembutal anesthesia. The abdominal skin was shaven and prepared with 70 per cent alcohol containing 1:5000 hyamine. A 15–20-mm. incision was made through skin, abdominal musculature, and peritoneum. The incision was retracted, and gentle manipulation of the intestines with blunt forceps was performed. The peritoneum was closed with #4-0 silk suture, and the skin was closed with a Michel skin clamp. This surgical procedure was repeated every 2 weeks until termination at the end of the 17th week.

All mice were housed, five or six per pen on wood shavings, fed Purina Laboratory Chow and water ad libitum, weighed every 2 weeks, and the carcinogen injection site was palpated weekly. When tumors appeared, they were measured with calipers in two diameters and recorded.

The time of appearance of tumors was determined by collating the palpable characteristics by the 14th week was greatest in the group subjected to laparotomy followed by the skin-wounded group; the control group manifested the smallest percentage of mice with early appearance of tumors. Toward the termination period, the differences in tumor incidence among the groups diminished, which is to be expected in view of the fact that the carcinogen dose employed ultimately induces tumors in 100 per cent of C57BL/6 mice (6).

Deaths with tumor occurred in the three groups of mice by the 17th week as follows: in the group subjected to laparotomy, 38 of 72 mice (53 per cent); in the group subjected to skin wounding, 23 of 82 mice (28 per cent); in the control group, 17 of 78 (22 per cent). Deaths with large, progressively growing tumors occurred earlier and were more numerous in the surgically treated mice than in the controls (Table 2).

The data reveal a quantitative relationship between the degree of severity of surgical trauma and time of tumor initiation and tumor growth.
rate. The more severe surgical trauma due to laparotomy resulted in more than twice the number of mice dying with large, progressively growing tumors as compared with the controls (53 per cent for the laparotomy group and 22 per cent for the controls). Deaths in the less severe surgical trauma of skin wounding group, although only slightly more frequent than in the control group (28 per cent compared with 22 per cent for the controls), did, however, occur earlier (Table 2). At the 12th week, tumors were present in 64 per cent of the mice subjected to laparotomy, in 41 per cent of the mice subjected to skin wounding, and in 22 per cent of the control mice. Of these, there

were no deaths in the control group until the 15th week, whereas five and seven deaths with large tumors occurred prior to the 15th week in the skin-wounded and laparotomized groups, respectively. It appears that the tumors in both surgically treated groups progressed at an accelerated rate compared with the tumors in the control group (Tables 1 and 2).

The effect of the surgical trauma on DBP tumorigenesis was not due to trauma-induced inanition. All mice were grouped at the start of the experiment on the basis of equal numbers per group based on weight, age, and sex. Mice were weighed every 2 weeks. In all three groups all mice showed a weight gain usual for C57BL/6 mice in the age employed. In comparison of weight data the presence and size (measurement) of tumors were considered. There was no gross evidence of inanition or obvious and severe loss of weight in any of the mice except at the terminal period with large progressing tumors prior to death.

**DISCUSSION**

The data clearly demonstrate that there is a significant effect by surgical trauma on the rate of tumorigenesis induced with a known chemical carcinogen. Since our data reveal that these effects are pronounced in the latent period of DBP tumor induction, attention is focused on the role of surgical trauma during the precancer stage in animals.

It appears that the surgical procedures employed in these experiments act as a co-carcinogen during the early carcinogenic period induced by the 3,4,9,10-dibenzpyrene. This question has important bearing on the tumorigenic process and needs to be investigated from this point of view with sub-effective doses of the carcinogen. These studies are in progress.

It is well known from clinical post-mortem data, supported by experimental animal data by Fisher and Fisher (2), that viable cancer cells exist in a dormant state. Recent reports indicate the existence of cancer-inducing agents such as "masked" viruses among others. It is of more than theoretical interest, therefore, to question and determine the role of surgical trauma on the "dormant" cancer problem. Experiments are under way in this regard on mice with the known presence of mammary tumor agent.

**ACKNOWLEDGMENTS**

We wish to acknowledge the assistance of Dr. F. Homburger and Mr. R. Kenney of the Bio-Research Consultants, Cambridge 41, Massachusetts for the method of tumor induction employed by them in their available 3,4,9,10-dibenzpyrene tumors in C57BL/6 mice.

**REFERENCES**

Effect of Repeated Surgical Trauma on Chemical Carcinogenesis

Bernard Gottfried, Norman Molomut and Joseph Patti


Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/21/5/658

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.