Studies on the Prevention of Surgical Implantation of Cancer *

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DESPITE the increased breadth of extirpative surgery in recent years, the local recurrence of cancer has remained a major obstacle in the control of malignant disease. While this type of persistent disease usually has been explained by incomplete excision of the primary neoplasm, in many instances tumor may recur within the wound in an area quite remote from either the primary site or regional lymph nodes. This finding suggests the possibility of there being other responsible mechanisms. Such a mechanism may be direct tumor implantation.[1, 10, 14] In contrast to normal cells which readily adhere to one another and are restricted in their movement, the neoplastic cell is characterized by a lack of mutual adhesiveness that apparently is progressive as the tumor becomes more anaplastic.[2, 6] As a consequence, the trauma of operation not only increases systemic dissemination of cancer through blood and lymph vessels but also enhances direct loss into the operative wound and body cavities. In those operative procedures in which the neoplasm erodes an epithelial or mesothelial surface and in those in which the neoplasm is incised accidentally or for biopsy, fragments of tumor frequently contaminate the surgical wound. Wound implantation by these viable free tumor cells may be a controllable factor in local recurrence of cancer.

The concept of "seeding" cancer cells into surgical wounds is an old one. In 1867, only nine years after the publication of Virchow's "Die Cellular Pathologie," Moore[15] made specific reference to this hazard in commenting upon the surgical treatment of breast cancer:

> "In the performance of the operation it is desirable to avoid not only cutting into the tumor but also seeding it; no actual morbid structure should be exposed lest the active microscopic elements in it should be set free and lodge in the wound. Diseased glands should be taken away by the same dissection as the breast itself without dividing the intervening lymphatics."

Evidence that inoculation of viable neoplastic cells occurs and may result in recurring cancer comes from three sources: (1) demonstration of neoplastic cells in washings from instruments, surgical gloves and operative wounds in patients undergoing cancer operations;[4, 17, 20] (2) well-documented instances of tumors being transplanted to sites separate or remote from the area of the primary tumor; and (3) high incidence of local recurrence in malignant diseases eroding epithelial or mesothelial surfaces.[10, 14, 16]

Just as recognition of the hazards of tumor contamination of the operative wound has been present for some time, so have efforts to control the growth of these potentially implanted tumors. Lack[12] was one of the earliest to employ preventive means, utilizing carbolic acid spray. At about the same time, Babler[3] was using alcohol, bichloride of mercury and hydrochloric acid in operative wounds. Mayo,[13] in 1913, employed 10 per cent formalin-soaked sponges for such a wound. Goli-

* Submitted for publication July 21, 1959.

Supported by Institutional Grant 324 AME 23-9 from the American Cancer Society.

gher[8] attempted to reduce the number of desquamated epithelial cells in the bowel lumen by mechanical washing with a dilute solution of perchloride of mercury before resection and anastomosis. More recently, irrigation of operative wounds with 0.5 per cent formalin,[19] Clorpactin XCB®,[5, 7] as well as 0.002 per cent nitrogen mustard[11] have been employed. To date, however, with the exception of Goligher's findings of a lower incidence of local recurrence at the suture line following appropriate washing, there has been no well-documented evaluation of the effectiveness of these measures as a means of controlling local implantation of cancer.

Experimental Studies

Since neoplasms frequently involve mesothelial or epithelial surfaces or require open biopsy, inoculation of the surgical wound is a necessary risk and there is a need for controlling this aspect of malignant disease. Principles of management of the experimentally-contaminated wound have been studied in laboratory animals. Initial investigations were concerned with control of Ehrlich ascites tumor following intraperitoneal inoculation of two to four million tumor cells.[17] Intraperitoneal nitrogen mustard administered within the first 48 hours usually "cured" from two-thirds to three-fourths of the animals. When treated at 72 hours, only from one-fourth to one-third of the animals survived without tumor. Treatment after that time only delayed development of ascites and time of death. These studies suggested that the "free" or "unestablished" tumor cell was highly susceptible to the chemotherapeutic agent employed. However, once invasion of the host tissues occurred, treatment resulted only in delay of tumor development. It was concluded that implantation and the implicit establishment of a host-tumor relationship enabled the tumor cell to survive a dose of chemotherapeutic agent that would be lethal for the unestablished cell.

This present study was undertaken to evaluate the effect of a number of agents on the control of Ehrlich ascites tumor implanted intraperitoneally or in the subpannicular areolar tissues.

Methods

Strain A mice of both sexes weighing between 25 and 30 Gm. were employed as hosts. Ascitic fluid was harvested from donor animals at six to eight days. In the first group of experiments, 30 to 60 minutes after the intraperitoneal inoculation of 24,000 to 36,000 cells in 0.2 cc. of ascitic fluid through a number 25 needle, one ml. of the agent to be tested was injected intraperitoneally. Mice were evaluated in terms of time of appearance of ascites, survival time and the development of a subcutaneous tumor. In the second group of experiments, mice were anesthetized with intraperitoneal sodium pentobarbital and a 2-cm. transverse incision made over the back. A 2-cm^2. "wound pocket" was made beneath the panniculus carnosus with dissecting scissors. Into this area, 24,000 to 36,000 tumor cells were instilled and the wound irrigated or sprayed with 5 cc. of an appropriate agent at various time intervals after tumor inoculation. All wounds were closed with clips. Nitrogen mustard, Clorpactin XCB®, chloramine-T, Lugol's solution, formalin, and benzalkonium were used as chemotherapeutic agents. Mice were killed by cervical fracture on the fourteenth day, the panniculus carnosus peeled back, and the wound appraised for gross amount of tumor as well as extent of dissemination.

Results

1. Effect of various chemotherapeutic agents administered intraperitoneally on the intraperitoneal growth of Ehrlich ascites tumor (Table 1). All agents studied showed evidence of tumoricidal effect. Clorpactin XCB® at concentrations of 0.5 and 1 per cent failed to prevent tumor

TABLE 1

Agent	No. of Mice	No. Developing Ascites Tumor	No. Without Ascites Tumor	No. With Tumor Implant of Abdominal Wall	No. Without Ascitic or Solid Tumor (%)
.9% saline	10	9	1	0	1(10)
.5% Clorpactin XCB®*	10	7	3	0	3(30)
1% Clorpactin XCB®*	33	19	13	7	7(21)
2% Clorpactin XCB®*	26	6	20	12	8(31)
.5% formalin	19	0	19	7	12(67)
.25% Chloramine-T*	10	2	8	2	6(60)
.5% Chloramine-T*	10	All dead without tumor within 4 days			
2 mg. % nitrogen mustard	10	0	10	1	9(90)
5 mg. % nitrogen mustard	18	2	16	0	16(89)
10 mg. % nitrogen mustard	10	All dead without tumor within 4 days			

* In .9% saline.

Results of intraperitoneal injection of 1 cc. of chemotherapeutic agent 30 to 60 minutes after intraperitoneal inoculation of .2 cc. ascites tumor (24,000–36,000 cells) in Strain A mice. Evaluation on 16th day.

growth and ascites in approximately 70 per cent of the animals. A 2 per cent concentration prevented ascites in 19 of 26 mice. However, in 12 of these, 2- to 3-mm. "tumor nodules" developed at the site of intraperitoneal puncture. Chloramine-T in concentrations tolerated by the mice (0.25%) was ineffective. Lugol's solution

TABLE 2

Agent	No. of Mice	No. Developing Tumor in Wound	No. Without Tumor in Wound	Percentage "Well"
Control (no treatment)	20	20	0	0
.9% saline	68	68	0	0
.5% Clorpactin XCB®	8	7	1	12.5
1% Clorpactin XCB®	25	18	7	24.0
2% Clorpactin XCB®	36	19	17	47.0
Benzalkonium 1:1000	10	6	4	40.0
.5% Chloramine-T	10	8	2	20.0
1% Chloramine-T	9	4	5	56.0
2% Chloramine-T	14	5	9	64.0
Lugol's 1:10	18	16	2	11.0
Formalin	14	8	4	29.0
2 mgm. % nitrogen mustard	19	16	3	16.0
5 mgm. % nitrogen mustard	47	5	42	89.0
10 mgm. % nitrogen mustard	10	0	10	100.0

Results of wound irrigation with 5 cc. of chemotherapeutic agent 30 minutes after inoculation of a 2-cm. wound beneath the panniculus carnosus with .2 cc. ascitic tumor (24,000–36,000 cells). Strain A mice employed as hosts. Evaluation on 14th day.

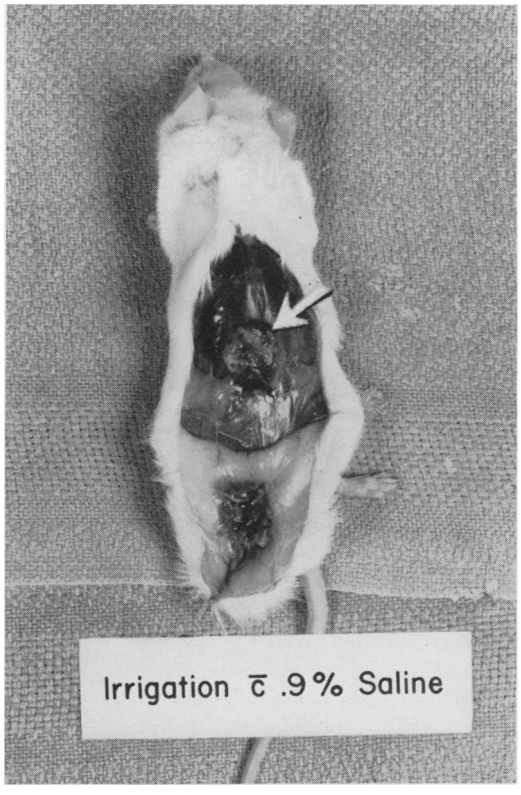

FIG. 1. Gross appearance of tumor 14 days after tumor inoculation of an experimental wound. The wound was thoroughly irrigated with 5 cc. saline 30 minutes after tumor inoculation.

was ineffective at concentrations not sufficiently toxic to cause death. Nitrogen mustard at a concentration of 5 mg. per cent prevented development of ascites in 18 of 20 mice. In contrast to animals receiving Clorpactin and formalin, none developed nodules at the site of peritoneal puncture. Control animals receiving 0.9 per cent saline all developed ascites and died prior to the eighteenth day.

2. Effect of topically applied chemotherapeutic agents administered 30 to 60 minutes after tumor inoculation of an experimental wound (Table 2). Control animals and those treated with 0.9 per cent saline developed tumors measuring 5 to 15 mm. in diameter (mean—10 mm.). (Fig. 1, 2). All of the agents used were cytotoxic in varying degrees; in general, those tumors developing in treated animals were one-fifth to one-tenth the size of those in control animals. The most effective agent was nitrogen mustard. Whereas 10 mg. per cent nitrogen mustard was 100 per cent effective, one mouse developed necrosis of the wound and delayed healing was evident in three others. Five mg. per cent nitrogen mustard caused minimal interference with wound healing and was 89 per cent effective. (Fig. 3, 4, 5, 6) In general, these results were in keeping with the cytotoxicity of these agents as evaluated against the intraperitoneal implantation of ascites tumor.

3. The effect of 5 mg. per cent nitrogen mustard administered topically at 1, 24, and 72 hours after tumor inoculation of the experimental wound (Table 3). At 1, 24,

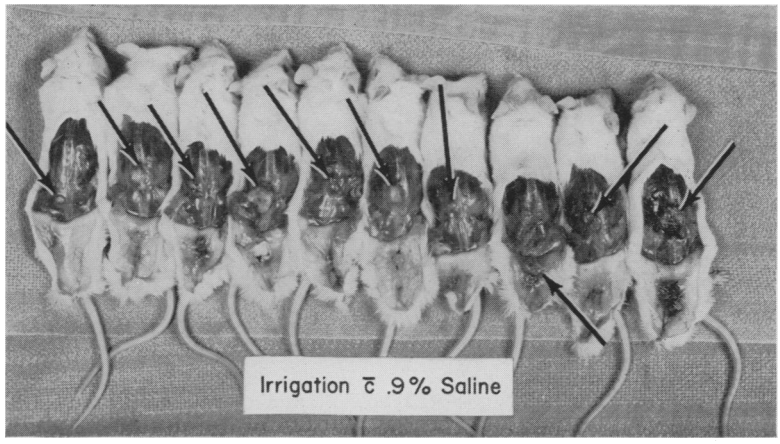

FIG. 2. Gross appearance of 10 mice 2 weeks after inoculation of ascites tumor into experimental wound and subsequent treatment by saline irrigation. Tumors varied in size from 3 to 11 mm.

and 72 hours, 5 mg. per cent mustard proved highly effective. That this effect was topical rather than systemic was confirmed by the observation that intraperitoneal administration of nitrogen mustard (2 mg./kg. body weight) was ineffective in control of the tumor where experimentally implanted in a subpannicular wound. Irrigation of an experimental wound one week after tumor inoculation was followed by persistent tumor in 12 of 20 animals studied.

Discussion

The ideal tumoricidal agent for prevention of tumor implantation in the surgical wound should be one causing maximum destruction of the free neoplastic cell with minimal interference with normal reparative processes of the host. Although such an agent could be administered either systemically or locally, it is probable that the latter would permit a higher local concentration of the agent and fewer over-all systemic effects. All of the agents herein studied are cytotoxic and it is likely that the rapidly proliferating neoplastic cell is more sensitive than the more slowly multiplying normal cells of the host. Pharmacologic actions of these drugs would seem to depend upon their ability to penetrate the cell membrane and then interfere with or damage intracellular enzyme systems. In no instance, however, is the mechanism of action well defined. Apparently complete destruction of all neoplastic cells is unnecessary since the resistance of the host may control tumor growth when the inoculum is small.

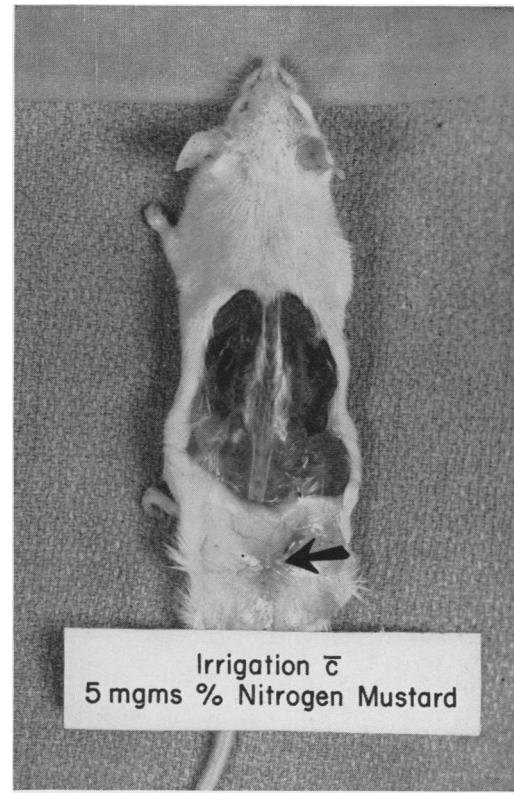

FIG. 3. Gross appearance of wound 2 weeks after tumor inoculation and subsequent treatment with 5 mg. % nitrogen mustard. Note the well-healed wound as well as the lack of inflammatory response involving postvertebral fascia.

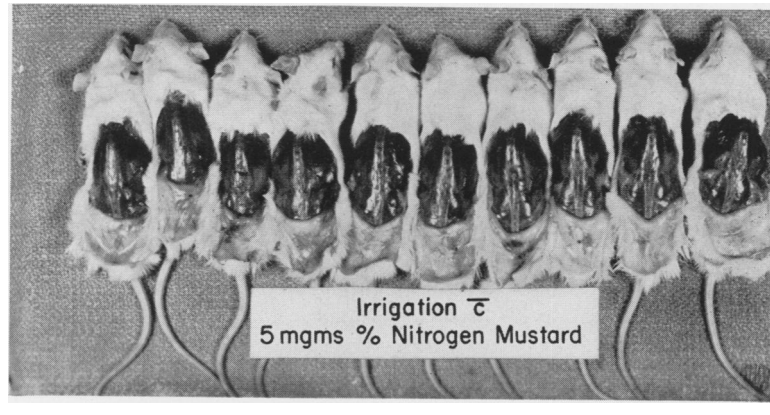

FIG. 4. Gross appearance of wounds of 10 mice 2 weeks after tumor inoculation of experimental wound and treatment with 5 mg. % nitrogen mustard.

TABLE 3

Time Between Tumor Inoculation and Chemotherapy	No. of Mice	No. Developing Tumor in Wound	No. Without Tumor in Wound	Percentage "Well"
30 minutes	47	5	42	89
24 hours	10	2	8	80
72 hours	10	0	10	100

Results of wound irrigation with 5 cc. of 5 mg. % nitrogen mustard at 30 minutes, 24 and 72 hours after inoculation of a 2-cm. wound beneath the panniculus carnosus with .2 cc. ascitic tumor (24,000–36,000 cells). Strain A mice employed as hosts. Evaluation on 14th day.

The action of both Clorpactin XCB® [21] and chloramine-T [9] is by way of the liberation of hypochlorous acid and its associated oxidizing potential. Both compounds were fairly effective as cancericidal agents by the criteria employed. The slower liberation of hypochlorous acid from Clorpactin XCB® may account for its decreased action for short periods, *in vitro* [17] and greater effectiveness in higher concentrations, *in vivo*. This mechanism may also be responsible for the lower toxicity of Clorpactin XCB®, *in vivo*, as compared with chloramine-T. Both of these agents have the disadvantage of being inactive in alkaline media and reacting with all organic matter.

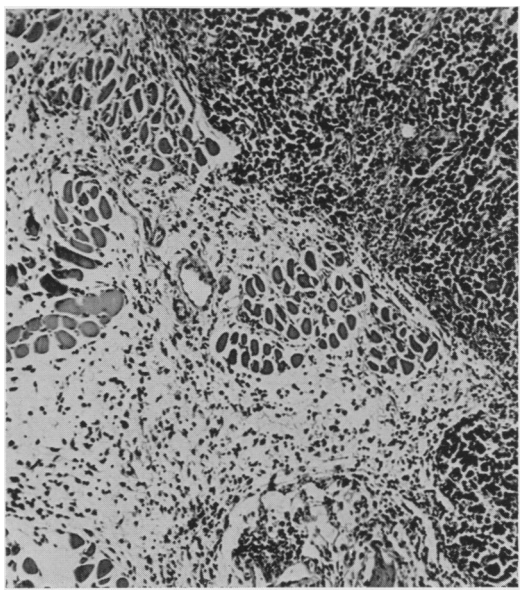

FIG. 5. Histologic appearance of tumor in wound 2 weeks after Ehrlich ascites tumor inoculation followed by irrigation with 2% Clorpactin XCB® (H & E × 100).

Lugol's solution with similar oxidizing properties was not effective in concentrations well tolerated by the animal. This is in keeping with studies disclosing a low cellular toxicity index (against leukocytes and embryonic tissue) despite a high bactericidal effect.[9]

Formalin exerts its cytotoxic action by way of its reactivity with proteins. In solutions which are antibacterial, it is highly irritating to human tissues. From these investigations, it is only moderately effective as a cytotoxic agent in concentrations tolerated by the animal.[9]

Nitrogen mustard in varying concentrations gave the most consistent tumoricidal effect with all methods of appraisal. Its cytotoxicity is related to ability to interfere with nucleic acid metabolism (primarily cessation of DNA synthesis).[9] Concentrations of this agent above 5 mg. per cent were highly tumoricidal in the previous, *in vitro*,[17] as well as in the present *in vivo* studies. Concentrations of 10 mg. per cent, however, when used locally produced necrosis of the skin in one mouse and evidence of delayed wound healing in others. In contrast to animals receiving chloramine-T and Clorpactin XCB®, there were no tumor nodules at the site of puncture wound of the abdominal wall. This finding suggests that the systemic effect of nitrogen mustard may have been of some value

in controlling tumor cells so implanted. It is unlikely that there would be any similar systemic effect from other agents used.

The factors which govern the successful growth of a potential tumor metastasis are quite complex [22] and may be completely unpredictable in a given patient. They concern not only the biological characteristics of the neoplasm but also the local environment at the site of potential implantation. Both these factors in turn are modified by the poorly-defined influence of "host resistance." It is most probable that just as most of the tumor cells that have been identified in peripheral blood fail to establish growing metastases, most tumor cells contaminating a surgical wound fail to survive. However, it is known that neoplastic cells do contaminate surgical wounds and that they are capable of survival and establishment of metastases. What is not known is the magnitude of the problem and how readily such potential implantation can be controlled.

In this experimental approach to the problem of "cancer seeding of the surgical wound," it must be emphasized that these studies concerned one particular tumor—Ehrlich ascites carcinoma, a hypotetraploid strain which has essentially a 100 per cent incidence of successful growth after subcutaneous, intraperitoneal or intravenous administration in the mouse. Furthermore, this tumor is highly susceptible to cytotoxic agents. Whether human cancer which may be somewhat similarily implanted in surgical wounds can be so controlled is unknown. Such studies are now in progress.

Summary

A review of clinical data strongly supports the role of the surgically-transplanted neoplastic cell as a possible cause for recurrence of cancer within operative wounds. The problem of wound contamination by viable and potentially-invasive neoplastic cells has been approached by studying the control of implanted Ehrlich ascites tumor in the experimental animal. The findings have suggested that the free or unestablished cell is highly susceptible to an appropriate chemotherapeutic agent. Once implantation and invasion of peritoneal or wound surfaces have occurred, however, this susceptibility markedly diminishes so that only palliative benefits result. The most effective tumoricidal agent that was also compatible with "normal" wound healing was 5 mg. per cent nitrogen mustard.

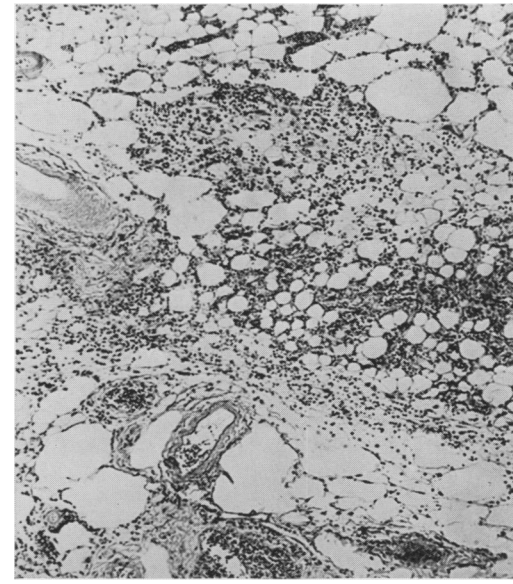

FIG. 6. Histologic appearance of wound 2 weeks after Ehrlich ascites tumor inoculation followed by irrigation with 5 mg. % nitrogen mustard. Although a number of chronic inflammatory cells remain, no tumor cells were observed (H & E × 100). See Figure 5.

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