Tumor Cell Contamination of the Surgical Wound: *

Experimental and Clinical Observations

COLIN G. THOMAS, JR., M.D.

From the Department of Surgery, and North Carolina Memorial Hospital, University of North Carolina School of Medicine, Chapel Hill, North Carolina

A MALIGNANT NEOPLASM is characterized by its ability to invade as well as to establish metastases by way of the blood and lymphatic systems. These biologic characteristics, seem to be related to 1) a loss of "adhesiveness" of the neoplastic cell; [5] 2) an inherent ameboid activity of the isolated cell; [9] and 3) the ability of separated cells to implant and establish themselves in a new environment, a complex phenomenon involving both nutritional and immune factors. Some of these properties may be accentuated as a tumor becomes more anaplastic.[2] As documented by the finding of neoplastic cells in the blood, lymph, and body cavities, it is these same characteristics that are probably responsible for carcinomatous cells being disseminated during the lifetime of a tumor.[8, 21, 27] The distribution of these cells is in accordance with anatomical factors of blood supply and blood flow.[6] Their survival and continued growth depends upon site of arrest and ability to implant. In addition, however, tumor dissemination may be enhanced during the trauma of extirpative surgery. Recently cancericidal agents have been used in conjunction with surgical treatment in an attempt to control this aspect of tumor spread.[27, 29, 30]

Additional hazards of cancer surgery are accidental incision of a tumor, incision of lymphatics containing cancer and direct dissemination of surface tumors. Thus local wound contamination as well as systemic spread of the disease may be a consequence of surgical treatment. The exact frequency of such contamination is unknown, although it would appear to be almost obligatory in neoplasms involving epithelial or mesothelial surfaces. Examination of wound washings has indicated an incidence as high as 26 per cent.[13, 33] Similarly, tumor cells have been recovered from gloves and instruments used during operative procedures.[4, 31] Although the relationship of these cells to the local recurrence of cancer is not clear, there is some evidence that the finding of such cells in a wound is associated with a higher incidence of recurrent disease.[16] This is in keeping with the observation of a relatively high incidence of local recurrence in those malignant diseases involving epithelial surfaces.[1, 17, 24, 28]

Because of this known occurrence of tumor cell contamination of the operative wound and its possible relationship to the local recurrence of cancer we have been concerned with methods of controlling the implantation of cancer in surgical wounds.

Factors Favoring Implantation in the Surgical Wound

Examination of the development of metastases using the transparent ear-chamber technic discloses that after tumor cells become adherent to a vessel wall they are immediately surrounded by platelets and a local thrombus is established.[35] The neoplastic cell protected by this thrombus now

* Presented before the Southern Surgical Association, Boca Raton, Florida, December 6–8, 1960.

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

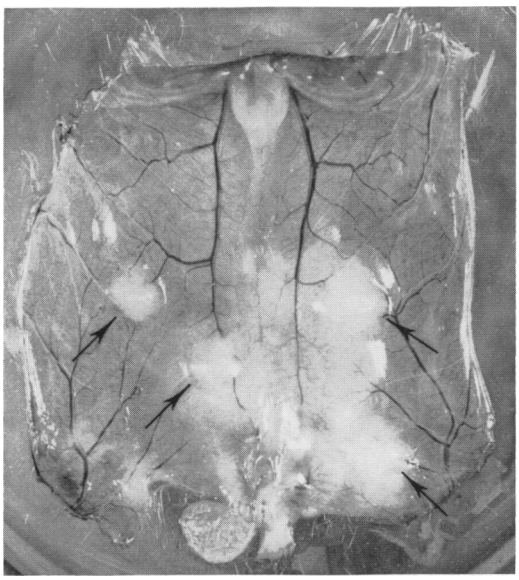

FIG. 1. The peritoneum of a mouse illustrating the propensity for tumor growth at site of injury. The upper abdominal quadrants were pierced with a No. 25 needle and the lower abdominal quadrants with a No. 20 needle. Thirty minutes later 0.1 ml. of Ehrlich ascites tumor was inoculated intraperitoneally. Examination on 10th day.

replicates and invades the vessel wall. In the absence of a protective clot there is little evidence of invasion. The surgical wound with its abundance of platelets, red blood cells, fibrin and tissue substrates presents an excellent site for implantation by the neoplastic cell. This can be nicely documented in the experimental animal.[25] In the mouse, the injection of two to three million Ehrlich ascites tumor cells is required for the establishment of pulmonary metastases. In contrast, implants in the peritoneal cavity can be established after the intraperitoneal injection of from 300,000 to 500,000 cells. One of the earliest sites of implantation is the area of peritoneal puncture demonstrating the favorable environment created by the wound (Fig. 1). The least number of cells is required when a fresh wound is inoculated.[18] Thus, the inoculation of a wound with 100 cells is followed by a tumor incidence of 27 per cent whereas an 81.1 per cent incidence is obtained with 1,000 cells. Modification of the host response by the administration of cortisone, by preliminary treatment with nitrogen mustard or by stress of an operative procedure may lower the number of cells required in each instance.

Since thrombus formation may be essential to implantation by the neoplastic cell, prevention of clot formation by the use of fibrinolysin should result in a lower incidence of metastases. This appears to be true.[12]

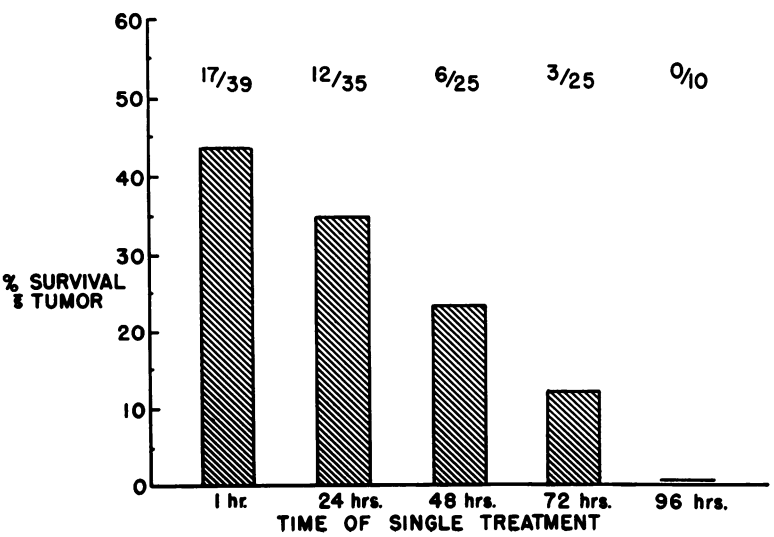

FIG. 2. Graph illustrating the relationship between time of topical treatment with nitrogen mustard and therapeutic result. Strain A mice were treated at periodic intervals after Ehrlich ascites tumor inoculation of the peritoneal cavity. The decreasing incidence of "cure" corresponds with histological evidence of invasion by the tumor.

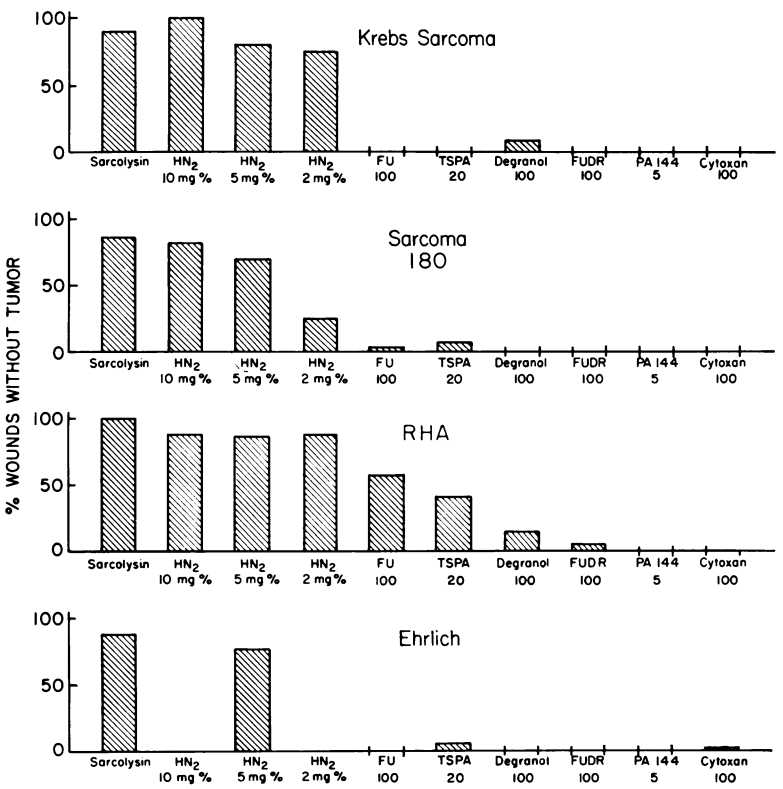

FIG. 3. Results of chemotherapeutic agents used in topical application against experimentally implanted cancer.

Relationship Between Implantation and Invasion and the Susceptibility of the Neoplastic Cell to Cytotoxic Agents

Experimental evidence indicates that the free or unestablished tumor cell is highly susceptible to an appropriate chemotherapeutic agent. Following inoculation of the peritoneal cavity with Ehrlich ascites tumor cells, the longer the time interval between inoculation and treatment the poorer the therapeutic result [22] (Fig. 2). Histological studies disclose that the cell is "implanted" on serosal surfaces during this peiod of susceptibility. Once there is evidence of invasion, however such topical treatment results only in an increased survival time not cure. This susceptibility of the free cell has been demonstrated for cells liberated into 1) closed tissue spaces; 2) open wounds; [34] 3) serous cavities; [22] and 4) the systemic or portal circulations.[29]

Topical Chemotherapeutic Agents in Experimentally Contaminated Wounds

An ideal topical chemotherapeutic agent should have a broad cancericidal effect with minimal interference with the normal reparative processes of the host. Although a high concentration of an agent may be achieved by topical application, the exposure period is brief and its effectiveness may not necessarily be predicted from the known systemic effect. This problem was studied by creating a standard experimental wound in Strain A mice of both sexes, and instilling 500,000 Ehrlich tumor cells in 0.1 milliliters of ascitic fluid. Thirty minutes later the wound was irrigated with an appropriate agent and closed.

Initial studies indicated that the oxidizing agents (Clorpactin-XCB® and Dakin's solution) and protein precipitating agent (formalin) were less effective than some of

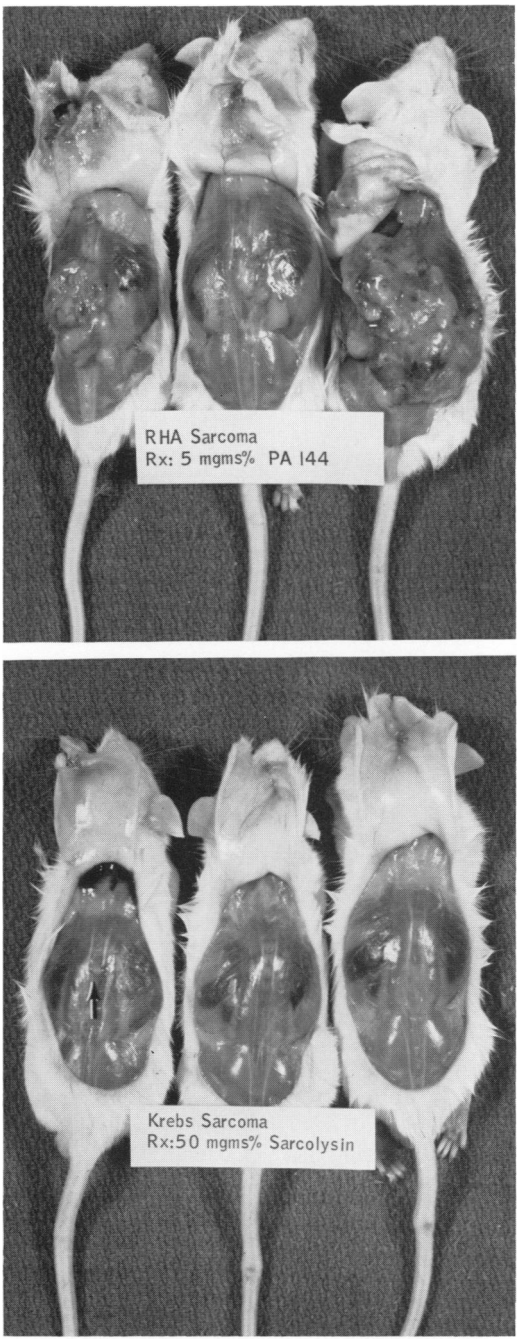

FIG. 4. Characteristic findings in mice showing (top) extensive tumor when PA-144 used as a topical agent and (bottom) absence of tumor when Sarcolysin was used.

the alkylating agents.[34] Current studies have involved a number of chemotherapeutic agents namely: 5-fluorouracil, 5-fluorodeoxuridine, mannitol mustard, (Degranol®), PA-144®, triethylenethiophosphoramide, Cytoxan®, nitrogen mustard, and phenylalanine mustard (Sarcolysin®). In addition to Ehrlich ascites tumors, the effects on three additional tumors have been evaluated, viz: Krebs sarcoma, Sarcoma 180, and a rhabdomyosarcoma (Fig. 3). All of these tumors may be grown in the ascitic form in Strain A mice and yield a solid tumor when implanted beneath the panniculus carnosus.

The efficacy of these agents can best be related to their mechanism of action. The antibiotic (PA-144) and antimetabolites (5-FUO, 5-FUDR) are thought to exert their effect by interference with cellular metabolic processes (DNA metabolism). For this reason, these agents require a longer time of action and could be anticipated to be ineffective in a single topical application. Similarly, those alkylating agents such as Cytoxan requiring a breakage of the phosphoramide linkage to become active prove to be of little benefit in topical application. The initial results with Sarcolysin appear to be good but are somewhat difficult to interpret because of the difficulty in procuring a satisfactory solution of this agent. Nitrogen mustard, which has a much more immediate and direct effect, was highly tumoricidal at both 5 and 10 mg.% (Fig. 4).

Effect of Nitrogen Mustard on Wound Healing

Systemic studies with nitrogen mustard in the experimental animal have indicated that only when the dosage was sufficient to cause nutritional depletion was there demonstrable decrease in wound healing. Measurements of wound tensile strength on the 8th postoperative day following wound irrigation with nitrogen mustard have indicated that concentrations in excess of 5 mg.% interfere with gain in tensile strength. There was a loss of 41 and 52 per cent with use of a 5 and 10 mg.% solution,

respectively.[15] By the fifteenth day, however, recovery was partially complete although the wounds had not attained normal tensile strength.

The above studies indicated that in the experimental animal using four different animal tumors, a chemotherapeutic agent was available that when introduced 30 minutes after tumor inoculation of a surgical wound, prevented tumor development in 75 per cent of the instances and was associated with only moderate interference in wound healing.

Clinical Observations

During the past three years the wounds of 125 patients undergoing surgical treatment of cancer of the oral pharynx, larynx, breast, gastro-intestinal and genitourinary tracts have been examined for the presence of neoplastic cells and subsequently treated with topical nitrogen mustard. After removal of the surgical specimen and the establishment of hemostasis the wound has been irrigated with 0.9 per cent saline using a DeVilbiss No. 135 atomizer. This fluid has then been aspirated, heparinized, centrifuged, and the resulting cell block studied for neoplastic cells. With such methods the over-all incidence of the demonstration of tumor cells has been approximately 20 per cent in neoplasms involving epithelial surfaces. The wound was subsequently irrigated using the same type of atomizer with 5 mg.% nitrogen mustard. Depending upon the size of the wound, between 25 and 100 milliters of the solution were employed. This fluid was permitted to remain within the wound. In intra-abdominal procedures 10 mg. of nitrogen mustard in a 5 per cent solution have also been deposited intraperitoneally through a small polyethylene catheter either at the time of operation or on the following day. These patients have been evaluated with particular reference to systemic and local effect of the nitrogen mustard, and the incidence of recurrent carcinoma. The results of such studies have been as follows:

1. **Systemic Toxicity.** As might be anticipated from the total amounts of nitrogen mustard used, no immediate systemic effect was manifest. Neither was there any late depression of leukocytes or platelets.

2. **Local Toxicity.** The local effects of these agents are more difficult to appraise and the associated problems of wound healing are cited. Of 43 *en bloc* and/or neck dissections for head and cancer, 34 wounds healed *per primam,* two developed wound infections and three developed a hematoma. Necrosis of a skin flap occurred in one patient and three developed an oro- or pharyngo-cutaneous fistula. Three wounds exhibited moderate edema or what might be considered a chemical cellulitis. Two deaths occurred in this group. An elderly patient with emphysema and asthmatic bronchitis underwent a laryngectomy and neck dissection. Postoperatively he developed a pharyngeal fistula, severe tracheo bronchitis and died of respiratory failure. The second patient died following radical excision of a carcinoma of tonsil and pharynx. Development of a large oro-cutaneous fistula contributed to her death from pulmonary infection and undernutrition.

When nitrogen mustard was used in association with 27 lesions of the gastro-intestinal tract, wound dehiscence occurred in six patients. Two patients failed to heal at the colon anastomoses sites and died of peritonitis. Wound infections developed in three.

In 14 patients undergoing radical mastectomy the wound healed *per primam* in 11. Fluid beneath the skin flaps, edema of skin flaps, and partial necrosis of a skin flap were each observed in one patient. No wound complications were seen in ten patients undergoing thoracotomy for carcinoma of the lung. In nine operative pro-

cedures on patients with genitourinary tract carcinoma there were no wound infections. One hematoma and one urinary fistula did develop in this group, however.

3. Local Recurrence of Cancer. The most important aspect of this study and the one that has been the most difficult to evaluate is the relationship of local recurrence of cancer to tumor cell implantation and its attempted control with topical nitrogen mustard. There are so many variables in these types of operative procedures that rather than attempt to compare similar groups of patients, the findings have been analyzed in terms of local recurrence with particular reference to extent of disease, adequacy of excision and chemotherapy.

In 43 patients with head and neck cancer, recurrence in eight was considered to be due to incomplete excision since tumor was present histologically at an excised margin. In seven other patients, however, excision appeared to be adequate by the same criteria. Wound recurrence developed in these individuals despite the use of topical nitrogen mustard as outlined. In five of these patients with local recurrence lymph nodes were positive for metastatic cancer. One patient is of particular interest in that a lymph node was ruptured during a neck dissection. Six months later multiple sites of recurrence were observed beneath the skin flaps.

In 14 patients undergoing radical mastectomy followed from six to 24 months (mean —16 months) no local recurrence was observed. Seven of these patients had lymph nodes involved by carcinoma. In 36 patients undergoing gastro-intestinal or genitourinary tract operations, one wound recurrence was noted. In two patients, however, diffuse peritoneal implants were noted approximately three and four weeks following the use of intraperitoneal nitrogen mustard. No wound recurrences were observed in ten patients undergoing thoracotomy for carcinoma of the lung.

Discussion

Although the concept of "seeding" cancer cells in surgical wounds is an old one, its incidence and role as a cause of recurrent cancer have been difficult to define. An increasing number of patients are being cited, in which the wound has been the site of metastaic focus.[1, 3, 4, 7, 10, 17, 24, 28]

In attempting to establish principles of management of tumor contaminated wounds by using homologous tumor transplantations in the experimental animal, one must remain keenly aware of the limitations of such methods. Factors of cellular immunity and host resistance are probably more important than in a spontaneous tumor in man and may provide a false impression as to the therapeutic effectiveness of various agents. Furthermore, there is little evidence that the suspceptibility of experimental animal tumors necessarily mirrors that of human neoplasms. In fact there is good reason to believe that there is not only considerable difference but that there is also considerable variation in the sensitivity of human tumors.

In the past numerous attempts have been made to control the growth of potentially implanted cancer in man. Carbolic acid spray, bichloride of mercury, hydrochloric acid as well as formalin soaked sponges have been used.[15, 34] More recent experimental studies have involved several different types of tumors in a variety of hosts as well as a number of chemotherapeutic agents.[15, 23, 34] In general nitrogen mustard has been most effective, although the oxidizing agents—Dakin's solution, Clorpactin XCB® and 0.5 per cent formalin—have received the widest clinical trials. To date, however, there have been relatively few attempts to appraise the results of such therapy. Goligher's finding of a lower incidence of recurrence at the site of anastomosis in colon cancer has been impressive.[11] Smith and Gehan, however, were unable to demonstrate any difference

in survival rates or incidence of local recurrence with the use of 0.5 per cent formaldehyde in the surgical treatment of cancer arising in the head and neck.[32]

The problem of attempting to evaluate the effectiveness of a local chemotherapeutic agent in a clinical setting is a complex one. It is most difficult to differentiate between local recurrence due to incomplete removal of the primary tumor and local recurrence on the basis of implantation metastasis. Topical chemotherapy would be of little value in the first instance. Furthermore, although the presence of tumor cells in the surgical wound may be disconcerting we do not know their true significance. It is quite possible that the number of cells required for gross identification in wound washings is quantitatively greater than the cells required for implantation metastasis. Under these circumstances, the presence of cells in the wound washings would be of little significance.

Although, the population sample is inadequate for a concise statistical appraisal, it is apparent from these experiences that the results of topical chemotherapy in man are not nearly as impressive as they are against experimental tumors in animals. Despite the liberal use of topical nitrogen mustard there were several patients in whom wound recurrence appeared to be on the basis of tumor implantation. This seemed to be fairly conclusive in two patients with peritoneal implants and in one patient with recurrence beneath cervical skin flaps following gross contamination by tumor. In addition, when these agents were used intraperitoneally in association with cancer of the gastro-intestinal tract the incidence of wound dehiscence was quite formidable. Although initial results may give an unfavorable impression as to the value of such agents, the principles of topical chemotherapy appear to be sound and such therapy may ultimately be an important adjunct to the surgical treatment of cancer.

The hazard of topical chemotherapy in man would appear to be related to total quantities of drugs used and any possible systemic effect in addition to local action. It is also possible an inappropriate agent might actually enhance local tumor growth as has been noted in the experimental animal with Clorpactin-XCB.[20] It is evident more information is needed on the significance of isolated tumor cells in surgical wounds. Only then can we judge whether possible benefits of topical chemotherapy will warrant hazards associated with use of these relative nonspecific cytotoxic agents.

Summary

Contamination of the surgical wound by the free tumor cell has been indicated as a significant and possibly controllable factor in the local recurrence of cancer. The problem has been approached by studying factors concerned with the successful growth of tumor in the experimentally contaminated wound. Prevention of tumor growth in such a wound can be accomplished in the experimental animal with an appropriate topical chemotherapeutic agent administered prior to invasion by the neoplastic cell.

The problem has been approached in patients undergoing radical cancer operation by irrigating their wounds with 5 mg.% nitrogen mustard. Despite such treatment recurrence interpreted as being on the basis of implantation was observed.

Bibliography

1. Ackerman, L. V.: Implantation of Cancer, An Avoidable Surgical Risk. Surgery, 37:341, 1955.
2. Ambrose, E. J. and G. C. Easty: International Cancer Congress. Science, 128:1512, 1958.
3. Boreham, P.: The Surgical Spread of Cancer in Urology. Brit. J. Urology, 28:163, 1956.
4. Brandes, W. W., W. C. White and J. B. Sutton: Accidental Transplantation of Cancer in the Operating Room with a Case Report. Surg., Gynec. & Obst., 82:212, 1946.
5. Coman, D. R.: Decreased Mutual Adhesiveness, a Property of Cells from Squamous Carcinoma. Cancer Res., 4:625, 1944.

6. Coman, D. R.: Mechanisms Responsible for the Origin and Distribution of Blood-Borne Tumor Metastases. Cancer Res., **13**:397, 1953.
7. Dockerty, M. B.: Pathologic Aspects in the Control of Spread of Colonic Carcinoma. Proc. Staff Meet. Mayo Clin., **33**:157, 1958.
8. Engell, H. C.: Cancer Cells in the Circulating Blood: Clinical Study on Occurrence of Cancer Cells in Peripheral Blood and in Venous Blood Draining Tumour Area at Operation. Acta Chir. Scand., Suppl. **201**:1, 1955.
9. Enterline, H. T. and D. R. Coman: The Amoeboid Motility of Human and Animal Neoplastic Cells. Cancer, **3**:1033, 1950.
10. Fortner, J. G. and W. Lawrence, Jr.: Implantation of Gastric Cancer in Abdominal Wounds. Ann. Surg., **152**:789, 1960.
11. Goligher, J. C., C. E. Duke and H. J. R. Bussey: Local Recurrence after Sphincter-Saving Excisions for Carcinoma of Rectum and Sigmoid. Brit. J. of Surg., **39**:100, 1951.
12. Grossi, C. E., D. Agostino and E. E. Cliffton: The Effect of Human Fibrinolysin on Pulmonary Metastases of Walker 256 Carcinosarcoma. Cancer Res., **20**:605, 1960.
13. Harris, A. H. and R. R. Smith: Operative Wound Seeding with Tumor Cells; Its Role in Recurrences of Head and Neck Cancer. Ann. Surg., **151**:330, 1960.
14. Harris, F. W. and C. G. Thomas, Jr.: Effect of Topical Nitrogen Mustard on Healing of the Experimental Wound. Surg., Gynec. & Obst., April, 1961.
15. Hatiboglu, I., G. E. Moore, H. Wilkens and F. Hoffmeister: Effects of Chemotherapeutic Agents on Wounds Contaminated with Tumor Cells: An Experimental Study. Ann. Surg., **152**:559, 1960.
16. Hilberg, A. W.: Presented before the American Society for Head and Neck Surgery, Chicago, 1960.
17. Hilberg, A. W., R. R. Smith, R. M. Miller, R. V. Eck, A. G. Ship and W. Kramer: Wound Seeding as a Cause of Failure in the Surgical Therapy of Cancer. Proc. Third Nat. Cancer Conf., Philadelphia, J. B. Lippincott, P. 568, 1956.
18. Jenkins, S. G., Jr. and C. G. Thomas, Jr.: Unpublished data.
19. Ketcham, A. S.: The Use of a Hypochlorous Acid Derivative in the Control of Cancer. Cancer, **13**:20, 1960.
20. Laszlo, J., D. Burk and K. Wight: Inhibition and Enhancement Effects of Hypochlorite on Ascites Tumor Cell Metabolism and Growth, and on Host Resistance. J. of Nat. Cancer Instit., **23**:351, 1959.
21. Long, L., O. Jonasson, S. Roberts, R. McGrath, E. McGrew and W. H. Cole: Cancer Cells in Blood. Arch. Surg., **80**:910, 1960.
22. McDonald, C. T., J. S. Howie, P. M. Weeks and C. G. Thomas, Jr.: Limiting Factors in the Prophylaxis of the Spread of Cancer at Operation by Chemotherapeutic Methods. Surg. Forum, **8**:164, 1958.
23. McDonald, G. O., S. M. Gines and W. H. Cole: Wound Irrigation in Cancer Surgery. Arch. Surg., **80**:920, 1960.
24. Meadows, C. T.: Surgical Transplantation of Tumor Cells. Am. Surg., **23**:247, 1957.
25. Moore, G. E.: Personal communication.
26. Moore, G. E. and T. Kondo: Study of Adjuvant Cancer Chemotherapy by Model Experiments, with Comments on National Clinical Adjuvant Chemotherapy Program. Surgery, **44**:199, 1958.
27. Moore, G. E., A. A. Sandberg and A. L. Watne: Spread of Cancer Cells and Its Relationship to Chemotherapy. J. A. M. A., **172**:1729, 1960.
28. Moore, O. S.: Surgical Failures in Cancer of Extrinsic Larynx, Pharyngeal Wall and Base of Tongue. Proc. Third Nat. Cancer Conf., Philadelphia, J. B. Lippincott, p. 559, 1957.
29. Morales, F., M. Bell, G. O. McDonald and W. H. Cole: The Prophylactic Treatment of Cancer at the Time of Operation. Ann. Surg., **146**:588, 1957.
30. Mrazek, R., S. Economou, G. O. McDonald, D. P. Slaughter and W. H. Cole: Prophylactic and Adjuvant Use of Nitrogen Mustard in the Surgical Treatment of Cancer. Ann. Surg., **150**:745, 1959.
31. Saphir, O.: Transfer of Tumor Cells by Surgical Knife. Surg., Gynec. & Obst., **63**:775, 1936.
32. Smith, R. R. and E. A. Gehan: Effect of Formaldehyde Wound Wash on Development of Local Wound Recurrences. J. of Nat. Cancer Instit., **23**:1339, 1959.
33. Smith, R. R., L. B. Thomas and A. W. Hilberg: Cancer-Cell Contamination of Operative Wounds. Cancer, **11**:53, 1958.
34. Thomas, C. G., Jr. and B. C. Brown: Studies on the Prevention of Surgical Implantation of Cancer. Ann. Surg., **151**:581, 1960.
35. Wood, S., Jr.: Pathogenesis of Metastases Formation Observed In Vivo in the Rabbit Ear Chamber. Arch. Path., **66**:550, 1958.