It is of interest to note that serum of non-immunized mice infected with tumor cells showed less beta and more gamma globulin protein than the ascites fluid obtained from the same group of mice. In contrast, the serum of immunized mice infected with tumor cells showed more beta and less gamma globulin protein than the ascites fluid obtained from the same pool of mice.

It is apparent that by suitable fractionation of ascites fluid large amounts of purified antibody, if not properdin, can be obtained for study of mouse serologic reactions.

*Summary.* Ascites fluid induced in mice by intraperitoneal injection of Ehrlich tumor cells contained specific antibody to *S. typhimurium* when mice were immunized against this organism. The serum and ascites were identical in their anticomplementary properties and neither contained detectable complement. The serum had normal properdin levels, but the ascites did not contain detectable properdin under the assay conditions employed.

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## Effects of Nitrogen Mustard N-Oxide, X Rays, and Cortisone on Yoshida Sarcoma Metastasis Development. (25258)

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At present, the most effective treatment for cancer is complete removal of malignant tissue by means of surgery. Despite the great value of this method, however, its usefulness is unfortunately limited by the difficulties involved in locating metastases. This particular limitation does not apply to carcinostatic agents, since their effects are systemic. Nevertheless, the mode of action of such therapeutic agents is but poorly understood, and many problems remain to be solved. For example, we have observed that the use of carcinostatic agents has sometimes been followed by an increase in tumor size or by the production of metastases, thus multiplying the problems connected with surgery.

*Materials.* An outbred strain of Gifu male rats weighing about 100 g was used in this investigation. Eight million cells of a 7-day-old Yoshida ascites tumor were transplanted subcutaneously into the right axillary area. Three weeks after tumor implantation, the animals were autopsied and examined for metastases. Certain batches of injected cells were taken from a tumor which had been made resistant to the carcinostatic effects of nitrogen mustard N-oxide ("NMO").† This tumor, which was kindly supplied by Dr. I. Hirono(1), had been made resistant to NMO

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† The NMO used was supplied under trade-name NITROMIN by Yoshitomi Pharmaceutical Industries, Osaka, Japan. The chemical name for this brand of NMO is methylbis(2-chlorethyl)amine N-oxide hydrochloride.

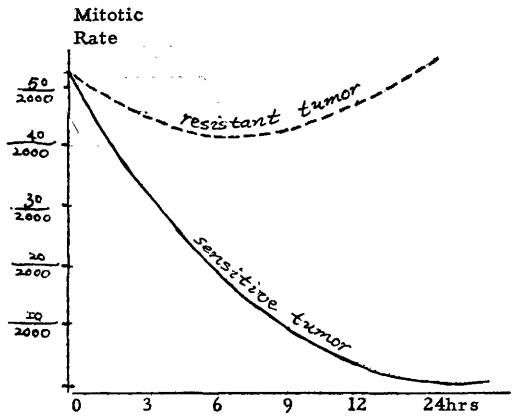


FIG. 1. Effect of NMO on the aseites tumor cell.

as follows: After tumor transplantation, subcutaneous doses of 50γ of NMO were given daily. The effects of this dosage on survival time, cell count, and mitotic rate gradually diminished to nil. After each cessation of effect, the dosage was increased, first to 100γ, then to 250γ, and finally to 500γ. About 70 to 100 days were required in order to develop a tumor resistant to a daily dosage of 500γ of NMO. Resistance of a tumor to NMO was tested by injecting a dose of 5 mg/kg body weight. The effect of this dose on mitotic rate is shown in Fig. 1. When the tumor is sensitive, the mitotic rate falls consistently, and the tumor does not grow. When the tumor is resistant, however, the mitotic rate temporarily undergoes a slight change, cell division being abnormal 3 and 6 hours after NMO injection, but recovering after 9 hours. In the absence of NMO, the results of transplanting NMO-sensitive and NMO-resistant tumors are essentially identical. When 5 mg/kg/day of NMO is administered for 21 days, however, a subcutaneously implanted resistant tumor containing 8 x $10^6$ cells enlarges to as much as 1.5 x 1.2 cm.

*Results.* Four general classes of treatment were studied: A. NMO for NMO-sensitive tumors; B. NMO for NMO-resistant tumors; C. X rays; D. cortisone. Each general class was divided into 4 specific types of treatment: 1. implantation of tumor cells, without treatment (controls); 2. treatment begun one week *after* implantation of tumor cells, and continued for one week; 3. treatment begun one week *before* implantation of tumor cells, and continued only for that week; 4. treatment begun one week *after* implantation of tumor cells, and continued for two weeks, till autopsy. The effects of the 16 individual types of treatment are summarized in Table I.

*A. NMO for NMO-sensitive tumors.* NMO was given in a dosage of 5 mg/kg/day. Types 1 and 4 resulted in only slight metastases, but types 2 and 3 produced remarkable ones. The results of type 2 suggest that the development of metastases is promoted by amounts of NMO insufficient to be carcinostatic. The results of type 3 demonstrate that the effect of NMO on the host is an important factor in the production of metastases. The results of type 4 indicate that the development of metastases can be inhibited by sufficiently large amounts of NMO.

*B. NMO for NMO-resistant tumors.* The dosage of NMO was the same as for NMO-sensitive tumors. All 4 types gave results similar to those produced by type A1. Such results indicate that NMO-resistant tumors have a limited capacity for forming metastases, regardless of the specific type of treatment within this general class. Since the effect of NMO on the host is presumably the

TABLE I. Effects of NMO, X rays, and Cortisone on Metastasis Development.

| | Nitrogen mustard N-oxide | | | | | | X rays | | | Cortisone | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sensitive tumor | | | Resistant tumor | | | | | | | | |
| Type | No. of rats | Tumor | Metastases | No. of rats | Tumor | Metastases | No. of rats | Tumor | Metastases | No. of rats | Tumor | Metastases |
| 1 | 11 | 2+ | 2+ | 11 | 2+ | 2+ | 11 | 2+ | 2+ | 11 | 2+ | 2+ |
| 2 | 12 | 3+ | 3+ | 8 | 2+ | 2+ | 8 | 2+ | 3+ | 12 | 2+ | 2+ |
| 3 | 12 | 3+ | 3+ | 12 | 2+ | 2+ | 8 | 2+ | 3+ | 18 | 2+ | 3+ |
| 4 | 12 | — | 1+ | 7 | 2+ | 2+ | 16 | — | 1+ | 10 | 2+ | 2+ |

Metastases: 1+, metastases to regional lymph glands and mediastinal lymph glands; 2+, metastases to abdominal lymph glands; 3+, metastases to various organs.

Tumor: —, none; 1+, less than 1 cm diameter; 2+, 1 to 2 cm diameter; 3+, more than 2 cm diameter.

same whether the tumor is sensitive or resistant, the phenomena produced by this class of treatment cannot be ascribed to host resistance. Evidently NMO-resistant tumors have little tendency to liberate cells into the blood stream.

*C. X Rays.* A dose of 100 r was given to the whole body every other day. Each type gave results essentially the same as those produced by the corresponding type in class A. These results indicate that the effects of X rays are closely parallel to those of NMO on an NMO-sensitive tumor. Whereas the NMO-sensitive tumors treated with NMO merely regressed, however, the tumors treated with X rays became necrotic.

*D. Cortisone.* The dosage was 50 mg/kg/day. Types 1, 2, and 4 gave results essentially the same as those produced by type A1, but type 3 produced remarkable metastases. These results suggest that cortisone does not promote the release of tumor cells into the blood stream, but does have a strong effect on the host.

*Discussion.* Metastasis production by means of cortisone was first reported by Agosin and his associates(2), and later confirmed by other investigators(3,4). Agosin's group concluded that this type of metastasis production is associated with inhibition of host resistance. Further consideration of this phenomenon has led to the development of "soil hypotheses" regarding susceptibility of the "soil." Metastasis production by means of local treatment with X rays has been explained as a transient local disturbance of the host-tumor relationship, probably facilitating entry of tumor cells into the blood stream(5-8). These reports suggest that chemical or physical carcinostatic agents have two types of effects on metastasis production: first, effects on the tumor itself, releasing tumor cells into the blood stream; second, effects on the host or on the organs invaded by the tumor.

The results of the present investigation support these conclusions. Evidently NMO has both types of effects on metastasis production, and so also do X rays. Cortisone, however, presumably has an effect only on the host. Considering the ability of carcinostatic agents to produce metastases when treatment is not continued long enough, it is apparent that clinical use of carcinostatic agents cannot be expected to provide satisfactory results if the doses employed are insufficient.

*Summary.* 1. If a subcutaneously implanted Yoshida sarcoma is sensitive to nitrogen mustard N-oxide ("NMO"), pre-treatment or short-time post-treatment with NMO produces remarkable metastases. The severity of these metastases following pre-treatment with NMO suggests a strong effect on the host. 2. Treatment of NMO-resistant tumors with NMO does not promote the development of metastases, even with pre-treatment or short-time post-treatment. This indicates that NMO-resistant tumors do not liberate cells to form metastases even when the host is under the influence of NMO. 3. Treatment of tumors with X rays produces essentially the same results as treatment of NMO-sensitive tumors with NMO does. In both instances, diminution of host resistance appears to be an important factor in promoting the development of metastases. 4. Pre-treatment of tumors with cortisone promotes the development of metastases; post-treatment, whether short-time or extended, has no such effect. Evidently the chief action of cortisone is on the host, increasing the ease with which metastases are formed. 5. Post-treatment with either NMO or X rays for an extended period inhibits the formation of metastases, and causes the tumor itself to regress. This observation suggests that carcinostatic agents should be used clinically only in large enough doses.

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