# Technical Papers

## Response of Irradiated Cancer Patients to *Vibrio metschnikovii*[1]

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Total body x-irradiation has been reported recently to inhibit antibody production in animals (*1–3*). However, since dosages used have frequently been in the neighborhood of the $LD_{50}$, results cannot be extrapolated to human beings undergoing therapeutic irradiation. It was reported by Evans (*4*) that lymphopenia-inducing irradiation of patients with neoplastic diseases caused a marked decrease in production of H agglutinins toward *Salmonella paratyphi.* However, the formation of H agglutinins would appear to be an extremely sensitive indicator, as evidenced by titers as high as 1: 65,536 following a single injection of *S. paratyphi* vaccine. It would therefore seem of interest to determine whether Evans' findings can be reproduced with a less sensitive system. The present investigation concerns responses to *Vibrio metschnikovii* by irradiated individuals with malignant neoplastic conditions. The fowl pathogen *V. metschnikovii* was chosen with the hope that native antibodies would not be present.

The vaccine employed was prepared from 4 day cultures of *V. metschnikovii* ATCC 7708 grown on the agar medium described elsewhere (*5*). Harvested cells were washed in saline, diluted to approximately 1 billion/ml, and killed by heating at 60° C for 1 hr. Phenol was added in a final concentration of 0.5%, and appropriate sterility and intracutaneous sensitivity tests were made. A series of 3 subcutaneous injections was given at weekly intervals, the first consisting of 0.5 ml and the remaining two of 1.0 ml each. Blood samples were drawn at the times of the first and last injections and at weekly intervals thereafter. Responses were measured by the growth agglutination technique reported earlier (*5*). Twofold dilutions of serum from 1: 2.5 through 1: 640 were made in the broth medium previously described (*5*) and inoculated with an 18–24 hr culture of *V. metschnikovii.* Readings were made after incubation for 18 hr at 37° C, the titer being the highest dilution of serum showing macroscopically visible agglutinated growth. Representative saline agglutination tests performed at 37° C with living cells gave comparable titers (*5*).

A total of 53 individuals was used in the study. Eleven were apparently healthy[2] and the remainder had a variety of malignant neoplastic diseases: chronic myeloid leukemia; Hodgkin's disease; lymphosarcoma; carcinoma of the breast; squamous cell carcinoma of skin, oral cavity, upper respiratory tract and cervix (Grades I–IV); and metastatic carcinoma. Diagnoses were established by conventional pathological methods. Of the 42 individuals with malignant neoplasms, 29 were receiving x-ray or radium therapy. Techniques varied from small volume irradiations for localized carcinomas to large abdominal portals over the spleen or retroperitoneal nodes, or total body irradiation. Integral doses ranged from 0.26 to 10.6 megagram roentgens, and therapy was usually administered over a period of about 2 weeks.

TABLE 1

DISTRIBUTION OF RESPONSES TO *Vibrio metschnikovii* VACCINE OF 29 IRRADIATED AND 13 NONIRRADIATED CANCER PATIENTS AND 11 NORMAL CONTROLS

| Individuals without Native Titer | | | |
|---|---|---|---|
| Maximum titer | Irradiated | Nonirradiated | Control |
| 5 | 1 | 0 | 0 |
| 10 | 0 | 1 | 1 |
| 20 | 1 | 3 | 1 |
| 40 | 2 | 1 | 0 |
| 80 | 3 | 2 | 0 |
| 160 | 4 | 1 | 0 |
| 320 | 0 | 0 | 0 |
| 640 | 2 | 0 | 1 |
| **Individuals with Native Titer** | | | |
| Fold increase in titer | Irradiated | Nonirradiated | Control |
| 2 | 2 | 0 | 0 |
| 4 | 5 | 2 | 1 |
| 8 | 2 | 1 | 0 |
| 16 | 0 | 1 | 1 |
| 32 | 4 | 0 | 0 |
| 64 | 1 | 1 | 2 |
| 128 | 2 | 0 | 2 |
| 256 | 0 | 0 | 2 |

Although to the authors' knowledge *V. metschnikovii* has not been isolated from man, over half of the individuals studied showed native antibodies. For these, fold increases of the maximum titers over original titers are shown in Table 1, along with maximum titers for individuals with no demonstrable native titers. Statistical analysis of both categories by means of the F test showed a high probability of homogeneity among the irradiated and nonirradiated groups and the few normal controls. Furthermore, no importance could be attributed to such factors as the integral dose (total amount of energy absorbed), time of irradiation relative to time of injections, site of irradiation, and type of malignant neoplastic process.

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[2] The authors gratefully acknowledge the technical assistance of Laura Lucilla Jenkins in securing some of the data on normal individuals.

However, in some individuals with metastases responses were somewhat less, irrespective of whether or not irradiation had been administered. This finding is in general agreement with earlier studies on the universal serologic reaction (*6*).

The data just presented failed to confirm the findings of Evans (*4*), who reported a marked depression of production of H agglutinins following heavy irradiation of individuals receiving a single injection of *S. paratyphi.* However, Evans' titers were in the order of $10^2$ times as high as our own, indicating a far more sensitive test system. Negative results in the present investigation may have been due to a masking of irradiation injury by a protracted course of immunization (*7*). Furthermore, it would appear that antibody production may not necessarily parallel resistance to infection (*8*). At any rate, the observed lack of effect on agglutinin formation would not appear to warrant a conclusion that immune defenses were in no way affected by irradiation.

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## Changes Induced by Indoleacetic Acid in Nucleic Acid Contents and Growth of Tobacco Pith Tissue[1]


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Studies in this laboratory on interactions of indoleacetic acid (IAA) with purines and pentoses in organ differentiation (*1*) suggested an effect of auxins on nucleic acid metabolism. To investigate this possibility, the effects of IAA on the nucleic acid contents of tobacco pith tissue were examined.

Stems were taken from 3- to 4-ft plants of *Nicotiana tabacum,* Wisc. 38, grown in a greenhouse. After leaves were cut off, the stems were cleaned thoroughly with a cotton wad wetted with 95% ethanol. The bark was then stripped from the stems, leaving a core of pith enclosed within a jacket of vascular tissue. Borings were made longitudinally through 3-cm segments of this pith core with a 7-mm diameter cork borer, and the cylinders of pith so obtained were cut into disks 3 mm thick. After the disks from several stems had been mixed together, they were planted on 50 ml of sterilized medium in 125-ml flasks, 5 to a flask. Sterile tissue cultures were obtained in this way without direct exposure of internal tissue to antiseptics.

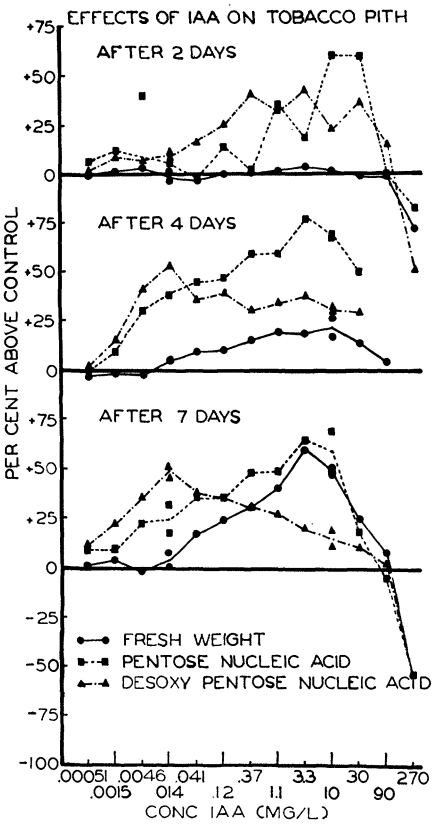


FIG. 1. Changes as per cent of controls in fresh weight, DNA content, and PNA content in excised tobacco pith tissue disks cultured on a sucrose agar medium with serial concentrations of IAA for 2, 4, and 7 days.

The increase in fresh weight of this pith tissue, cultured on an IAA-sucrose-mineral-agar medium, is a function of the supplied IAA over a wide concentration range (*2*). However, as the growth during the first 10 days is not enhanced by the presence of the mineral nutrients (*3*), a simplified medium, containing only Bacto agar, 1%; sucrose, 2%; and IAA was used for these experiments.

At regular intervals, duplicate samples of at least 5 disks each were taken and dispersed in cold 70% ethanol in a Potter-Elvehjem homogenizer. The homogenate was extracted by the method of Ogur and Rosen (*4*), which is a differential perchloric acid extraction procedure for separation of plant pentose nucleic acid (PNA) and deoxyribose nucleic acid (DNA). Quantities of nucleic acid were estimated by measuring optical densities of extracts at 258 mμ in a Beckman spectrophotometer, and they are reported as optical densities of PNA or DNA extracted from one

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