Role of Operative Stress on the Resistance of the Experimental Animal to Inoculated Cancer Cells *

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PRACTICALLY all surgeons have occasionally observed a rapid spread and growth of carcinoma shortly after an unsuccessful attempt to remove all of a carcinoma. This observation and experimental data accumulating in our laboratory recently, while conducting experiments on the "takes" of Walker 256 carcinosarcoma cells, have stimulated us to design an experiment to test out the hypothesis that operative stress might at times result in a decreased resistance of the host to cancer cells.

Materials and Methods

The present study was undertaken to evaluate the effect of surgical trauma, in the form of celiotomy in rats, on the "takes" of Walker 256 carcinosarcoma cells inoculated subcutaneously. The Walker tumor which we are using at the present time is so virulent that inoculation of a fresh saline suspension of 25,000 cells usually results in "takes" in 95 to 100 per cent of animals. In order to determine whether a given procedure will increase or decrease the percentage "take" of tumor cells, it is necessary that the "takes" in controls should be between 35 and 65 per cent. Experiments conducted by Haddon, Chan *et al.*[2] in our laboratory have shown that "aging" cells at room temperature for 12 hours will reduce the "takes" to a figure within those limits.

White, female, Holtzman strain rats weighing 180 to 200 grams were used. From each shipment of animals, the same number of controls were chosen as were utilized for the experiment. The cell suspension was made as indicated in the following paragraph.

The tumor of the donor animal was exposed under sterile technic with the animal under Nembutal anesthesia; portions of solid tumor were excised, placed on a glass shell, and, using sharp scissors, were subdivided minutely. These minced pieces were placed on a steel wire mesh (80 wires per inch) and sterile physiologic saline poured over them. A sample of the resulting cell suspension was counted in a hemocytometer, and the suspension diluted until it contained 25,000 cells per cc. The resultant cell suspension was then left standing at a constant temperature of 72° F. for 12 hours before being used.

All animals were anesthetized (30 mg. Nembutal per kilo of body weight intraperitoneally) and then divided into two groups for inoculation: (a) those having inoculation after celiotomy, and (2) those serving as controls after inoculation only. The backs of both groups of animals were shaved for the site of cell inoculation, and the abdomen of the celiotomy group shaved for celiotomy. After aseptic skin preparation, the abdomens of the celiotomy group were opened in rapid succession by means of a vertical midline incision. The abdominal contents consisting of small and large intestines, spleen and liver were then delivered through the incision and placed on the drapes. The abdominal viscera were

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left exposed in this manner for 45 minutes. During this period of time, the position of viscera on the drapes was manually changed every 15 minutes with the intent to subject them to the trauma of handling. Also, at ten minute intervals the exposed organs were sprinkled with physiologic saline solution to prevent drying.

At the end of 45 minutes the abdominal contents were replaced and the incision was closed in layers using a continuous suture of #40 cotton.

One cc. of tumor cell suspension (25,000 tumor cells in physiologic saline aged 12 hours) was then injected subcutaneously in the back of each animal using a tuberculin syringe and 25 gauge needle. To insure uniformity of time, the injections of tumor cell suspension were given alternately to the animals in the celiotomy and control groups.

It was thought that no less than 80 rats would be required for each group. Since no more than 15 or 20 rats for each group could be treated at one time, it was necessary to perform the experiment with five different groups of rats.

Animals were observed daily during the first postoperative week and on every third or fourth day thereafter. Part of the animals were sacrificed at 35 days and the rest at 90 days.

Results

The first discernible tumor usually appeared eight to 12 days after inoculation in the group having celiotomy; in the control group, the tumor appeared on the average about three days later. The tumors in the celiotomy group grew slightly faster than the controls.

Takes developed in 31.6 per cent of the control animals but in 61.1 per cent of the animals having celiotomy just before inoculation of cells (Table 1).

In general, the control animals with tumors lived longer than the animals subjected to celiotomy. In the control group, 75 per cent of animals developing tumors were alive at the end of 35 days compared to 41.7 per cent of animals in the celiotomy group. At the end of 90 days, 61.5 per cent of animals with tumors in the control group were alive compared to 33.3 per cent in the celiotomy group (Table 2).

The heaviest tumor in the celiotomy group at the end of 90 days following celiotomy was 115 Gm., compared to 80 Gm., in the control group. The weight of the animal with the tumor in the former instance was 355 Gm. and 315 Gm. in the latter.

TABLE 1. *Influence of Operative Trauma on the Takes of Walker 256 Cells Inoculated Subcutaneously*

Groups*	No. of Animals	Total No. of Takes	Per Cent Takes
Rats subjected to celiotomy	85	52	61.1
Controls	79	25	31.6

Each of the animals in both groups was inoculated with 25,000 cells aged 12 hours to reduce the takes considerably below 100 per cent.

TABLE 2 *Comparison of Survival Time in Animals Sustaining Takes With and Without Celiotomy*

	No. of Takes	Per Cent Alive at 35 Days	Per Cent Alive at 90 Days
Rats subjected to celiotomy	30	41.7	33.3
Controls	17	75.0	61.5

Discussion

Data from the experiments reported herein indicate that the trauma of celiotomy results in an increase in the "takes" of inoculated tumor cells, and to a slight extent increases their rate of growth; this presumably suggests that the operative stress resulted in a decreased resistance of the animal to the cancer cells. Other than the clinical impression mentioned earlier,

we have no information whatsoever on the role of operative stress on the growth of cancer cells in the human being. If a decreased resistance was induced by operation in the human being, we would expect it to occur only in certain patients and with certain tumors. At first thought, it might appear that this influence (if existing in the human being) would be a point against treatment of cancer by operation. However, the opposite would actually be true since we already know that surgery is the most effective method of treating cancer. If operation does reduce the resistance of some patients to their cancer cells, it might be possible to neutralize this deleterious effect and thus improve the results of operation.

Recent experiments of another type conducted by Lewis [3] in this laboratory suggest also that operative stress in animals may increase the tendency for the tumor to metastasize. He injected 100,000 T241 sarcoma cells into the hind foot of C57 mice, and on the 22nd day (several days after cells are known to metastasize to the lung with this tumor) amputated the extremity bearing the tumor. The number of animals having pulmonary metastases at death were recorded, and compared to the number of metastases in the control group having inoculation of cells but no amputation. In the group having amputation, pulmonary metastases were observed in 96 per cent compared to 57 per cent in the controls. The animals having amputation lived an average of 45 days, whereas the control animals lived 34 days. It hardly seems likely that the 11 extra days of life sustained by the amputated group could account for the marked difference in incidence of pulmonary metastases in the two groups.

In other experiments carried out in this laboratory by Chan and McDonald,[1] rats were subjected to the "stress' of a hepatotoxic chemical. Liver damage was produced by injection of carbon tetrachloride subcutaneously for three days prior to inoculation of 25,000 Walker 256 carcinosarcoma cells (aged 12 hours) into a branch of the portal vein. In the animals receiving carbon tetrachloride before inoculation of the cancer cells, takes in the liver were observed in 42.0 per cent compared to 18 per cent in the control animals having inoculation without carbon tetrachloride. We do not know whether chemical stress or the hepatic damage is the factor responsible for this difference in the percentage takes.

Summary

In this experiment testing the role of operative stress on the take and growth of Walker 256 cells injected subcutaneously into rats, takes were observed in 61.1 per cent of 85 animals subjected to celiotomy just before inoculation of cells, and in 31.6 per cent of 79 control animals inoculated with cells but not having a celiotomy. This suggests that the operative stress reduced the resistance of the animals to the inoculated Walker 256 cells. We do not know if this reaction is sustained by the human being. At first glance these data might appear to be a point against operative therapy, but on the contrary, if the reaction is sustained by the human being, we might improve our operative results by neutralizing the deleterious effect of the operation on host resistance. We already know that surgery is the most effective agent we have for the cure of cancer.

The control animals lived longer than did the animals having celiotomy. For example, at 35 days 75 per cent of the control animals were alive, compared to 41.7 per cent of the animals having celiotomy; at 90 days 61.5 per cent of the control animals were alive, compared to 33.3 per cent of the animals having celiotomy. The takes became palpable about three days earlier in the celiotomy group; likewise the tumor grew slightly faster and to a greater weight in this group.

Bibliography

1. Chan, Paul, G. O. McDonald and W. H. Cole: The Role of Hepatic Damage on Experimental "Takes" of Carcinoma. Submitted for publication.
2. Haddon, David, P. Chan and G. O. McDonald: The Influence of "Aging" of Cell Suspensions on the "Takes" of Walker 256 Tumors. To be published.
3. Lewis, Malcolm and W. H. Cole: The Experimental Increase of Pulmonary Metastases After Operative Trauma (Amputation of Limb with Tumor). Arch. Surg. In Press.

Discussion

Dr. J. Englebert Dunphy: I would like to compliment the speakers on these exciting and important contributions.

I would also like to compliment Dr. Scott and the Program Committee for seeing fit to place these four papers on the morning program. It is very fitting that he should have done so, because this society has always been interested in the biological behavior of cancer, and important contributions to this field have been made by Dr. Gatch, Dr. Harvey Stone, Dr. John Morton, and others, over the past years. It is a sure sign that surgeons are steadily and progressively lifting their heads above the simple concept of repetitive emphasis on early diagnosis and total, wide excision.

To be sure, this is indicated in appropriate cases, and lifesaving, but the reverse side of the equation is so great, and the number of patients who cannot be benefited by the old-time-honored approach so large, that these papers constitute a most important contribution to our thinking.

We have been interested in our laboratory, under the direction of Dr. W. Bradford Patterson, in the behavior of human transplanted tumors. One of the observations he has made is that they are more resistant to very deep freezing—that is, to within a few degrees of absolute zero—than normal tissue. Human cancer can be frozen without dehydration or the use of glycerol to within a degree of absolute zero, be replanted and grow. This may indicate a different enzyme behavior in human neoplastic cells. It is a field for further inquiry.

Dr. Patterson has also noticed that the behavior of tumors, when transplanted—the ability of a tumor to set up a reaction in the hamster varies tremendously. Certain tumors, when transplanted, appear to make the animal completely resistant to further transplantation of the same tumor, while other tumors can be repeatedly transplanted without an "immune reaction."

This phenomenon appears to be transplanted to the neighboring lymph nodes. If one excises the lymph node on the side of a hamster cheek pouch, after a tumor has gone through its growth cycle, and then transplants that node to the cheek pouch of another hamster with human tumor, the tumor immediately disappears. This does not occur if a lymph node from a "non-resistant animal" is transplanted.

(Slide) This simply shows the reaction at 4 days. This is a low-power view, and you see no essential reaction, lymp hnode and tumor growing side by side.

Here again, the same phenomenon—no reaction to the implantation. The tumor is taking successfully, and grows to reasonable size.

Here, with the node, a so-called "immune node," there is rapid destruction of tumor.

The significance of these observations, of course, we do not know; but again, it indicates that the tumor can set up some type of immune response or reaction on the part of the host which may have important implications in its ultimate growth potential.

Turning from the behavior of the tumor to that of the host, it is most important, I think, that we recognize that tumor can be destroyed by the human organism. We have described situations in which, in the presence of widespread metastasis the tumor has disappeared at one site while continuing to grow in another. This is an age-old observation. There must be some local factors involved.

We have attempted to explore this by implanting an animal tumor into tissue under different circumstances, such as into the healing wound, but if the tumor grows well—we have used the Walker 256—in subconscious tissues, it appears to grow equally well in various stages of the healing wound. It does not appear to grow faster or be inhibited by it.

Finally, a word about operative trauma during the procedure, as referred to by Dr. Cole. Our group are involved in the Adjuvant Therapy Study, and we have been disturbed by one or two patients in whom, following resection of the tumor, and the implantation of mustard into the peritoneal cavity, very widespread peritoneal metastases appeared within a very short time. This does not necessarily imply that tumor cells cannot be destroyed by the local use of chemotherapeutic agents. To me it suggests we must know more