Pre-excisional Fixation of Tissues in the Treatment of Cancer in Rats*

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A chemosurgical method for the removal of accessible neoplasms under complete microscopic control has been developed for use in human cancer. This is to be described elsewhere in the near future (2). The object of the present paper is to describe some of the animal research which led to the development of this method and which has been withheld from publication until the treatment had been applied clinically.

The basic idea was to devise a method by which cancers could be removed in successive layers, each of which could be studied systematically under the microscope to determine the presence or absence of malignant tissue. Such a technic would have the obvious advantage of enabling the removal of a given cancer with a minimal destruction of normal tissue.

The only practical and safe means of removing cancer tissues in layers required their chemical fixation in situ prior to excision. After such fixation incisions were made only through killed and fixed tissue thus avoiding all danger of spreading cancer cells over the cut surface. Moreover, the fixed, form-retaining specimens could be used for immediate frozen sections so that by systematic mapping of the area under suspicion in relation to anatomical landmarks, the exact location of residual areas of cancer could be determined. Further fixation was confined to the cancerous areas and the process was repeated until completely cancer-free tissue was reached.

Fixative preparations and method of testing.—The first necessary step was the selection of a satisfactory chemical fixative along with a suitable means for its administration. For purposes of testing, rats bearing transplanted Philadelphia I sarcoma, mammary sarcoma MS-2, Flexner-Jobling carcinoma, and carcinoma C-256 were used. Preliminary experiments showed that application of fixative solutions alone to the tumor surface resulted in insufficient penetration while intratumoral injection not only gave erratic penetration but was often fatal because of direct entrance of the medium into blood vessels. For deep penetration and accurate control the best procedure proved to be application of the fixative in the form of a paste.

The paste to be tested was applied to the tumor surface and covered with a cotton dressing held in place by adhesive tape which in turn was sewed to the skin to avoid loosening by teeth or claws. After 24 hours most of the fixed layer of tissue was excised. Since the incision was made through fixed tissue in which all vessels had been thrombosed no bleeding occurred unless the level of incision was inadvertently carried too deep. If bleeding was encountered it was promptly controlled by applying a small square of fixative-impregnated gauze under momentary pressure. If further action of the fixative was required another application of the paste was made to the surface exposed by the first excision.

A number of chemicals were tested not only for ability to produce satisfactory fixation for microscopic detail but also for toxicity, ability to diffuse into tissues, and controllability in regard to depth of penetration. Caustic alkalies, such as sodium or potassium hydroxide, produced liquefaction of tissues with complete loss of structure. Arsenic trioxide, phenol, and mercuric chloride were too toxic, while silver nitrate carried the danger of argyria. With antimony chloride the fixed tissues sometimes separated too rapidly making excision of layers of tissue difficult; moreover this chemical strongly affected normal skin requiring extraordinary care for its safe use. The most satisfactory fixative from every standpoint was zinc chloride, a chemical first extensively used in the treatment of cancer over a hundred years ago by Canquoin (1) and more recently in the treatment of inoperable breast cancer by Strobell (3). This substance in a proper base produced adequate fixation, penetrated well, was thoroughly controllable, produced no general toxicity, and was safe to handle since it has relatively little effect on the intact skin.

For accurate control of fixation over a wide range of depths it was found to be essential that the base carrying the fixative have certain special characteristics; namely, a low affinity for the fixative solution.

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and a capillary structure. The low affinity for the fixative permits the chemical to leave the paste readily in favor of the tissues, while proper capillary structure enables the fixative to pass from all levels of even very thick applications, thus making it possible to vary penetration from less than 1 mm. to more than 1 cm. by simply altering the depth and area of application.

These properties may be obtained by certain combinations of "permeant" and "agglutinant" substances. The permeant is a finely granular material insoluble in the fixative solution and having a specific gravity of 4 or more; examples are pyrolusite, clinkers from bituminous coal, stibnite, limonite, hematite, ferric oxide, and ferric lactate. The agglutinant may be any of a variety of plant extracts such as *Sanguinaria canadensis, Phytolacca decandra, Podophyllum peltatum*, or *Inula helenium*. More common agglutinants such as starch or wheat flour are somewhat inferior but rye flour is fairly satisfactory. The agglutinant is used in just sufficient quantity to keep the fixative solution from separating from the base as it stands in the jar. The permeant supplies multiple interfaces making for a capillary structure; it also has low affinity for the fixative and along with the agglutinant gives body to the preparation.

The fixative is usually added in saturated solution. Zinc chloride should make up 40-50 per cent of the preparation by weight. A satisfactory preparation is the following:

- Stibnite (80 mesh sieve) ............ 40.0 gm.
- *Sanguinaria canadensis* ............. 10.0 gm.
- Zinc chloride, saturated (4:1) solution ... 34.5 cc.

In this preparation (Z-108a) zinc chloride makes up 45.2 per cent of the weight.

**Histology of tissues fixed in situ.**—All of the fixatives tried with the exception of the strong acids and alkalis produced good enough fixation for easy diagnosis by one acquainted with the alterations in cell structure produced by each fixative. The dehydrating effect of zinc chloride caused some cell shrinkage with resulting increase in density of cytoplasm, pyknosis, and increased basophilism of nucleus and the appearance of clefts between the cells, but with experience the histological structure of the tissues could easily be recognized (Fig. 1). Antimony chloride caused the nuclei to become swollen and vesicular, and with too strong action, produced an amorphous basophilic mass useless for diagnosis. Phenol caused moderate shrinkage and distortion. Mercuric chloride and silver nitrate produced excellent fixation. The usual freezing or paraffin embedding technics were used followed by hematoxylin-eosin staining.

In the normal tissues immediately surrounding an area of fixed tissue there develops after 24 hours a leukocytic infiltration which by 48 hours becomes a well defined wall of leukocytes. In this zone tissue dissolution occurs with resulting separation of the fixed tissue a few days later.

The leukocytic infiltration which is so prominent between fixed and unfixed normal tissues is almost entirely absent at the junction between fixed and unfixed malignant tissues. Because of this circumstance neoplastic tissues are rarely obscured by collections of leukocytes, hence diagnosis is easier.

**Effect of chemical treatment on metastasis.**—To test the possibility that the reactions set up in cancer tissue by treatment with fixative chemicals might increase the tendency to metastasis, repeated subcurative doses of zinc chloride were applied to a transplantable rat carcinoma. Flexner-Jobling carcinomas ranging in size from 2 to 4.5 cm. in diameter were paired off in equal sizes in the treated and control groups. In 4 experiments the treated tumors were injected every other day with 0.1 cc. of an 8-25 per cent zinc chloride solution, while the controls were untreated except for 1 group which received physiological saline solution. In 2 other experiments treatment consisted of daily applications of a small amount of a 40 per cent zinc chloride paste. Such subcurative doses were administered over a period of from 4 to 8 weeks. Rats dying during this period were examined for axillary, pulmonary, and mediastinal metastasis as were the rats killed at the termination of the experiment.

![Fig. 1.—Flexner-Jobling rat carcinoma fixed in situ with zinc chloride in upper half (A) and in vitro with Bouin's solution in lower half (B).](cancerres_aacibjournals.org)
A summary of the results of these experiments is given in Table 1. The failure of attempts purposely to increase metastasis by prolonged subcurative chemical treatment is evident. It indicates that certainly the rapid removal of tumors by chemical means could have no such tendency.

**TABLE 1: EFFECT OF REPEATED SUBCURATIVE DOSES OF ZINC CHLORIDE ON THE INCIDENCE OF METASTASIS OF FLEXNER-JOBLING CARCINOMA**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treated animals</th>
<th>Control animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. rats</td>
<td>Metastases</td>
</tr>
<tr>
<td>Injection of 8 to 25% solutions</td>
<td>39</td>
<td>9</td>
</tr>
<tr>
<td>Application of 40% paste</td>
<td>22</td>
<td>8</td>
</tr>
<tr>
<td>Total, both methods</td>
<td>61</td>
<td>17</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Because of the wide difference between the behavior of transplantable tumors and the available spontaneous neoplasms as they occur in animals, and the behavior of the usual accessible neoplasms encountered in humans, no serious attempt was made in these experiments to compare the efficacy of chemosurgical treatment with that of surgical or irradiation treatment. The expansive rather than invasive type of growth, the encapsulation, the easy visualization, and the lack of small caliber, unexpected outgrowths exhibited by the usual animal tumors, make them easily removable either by chemical or moderately wide surgical extirpation without the necessity of microscopic control.

Comparison with other methods had best be made upon specific human neoplasms. This is being done in a clinical paper.

**SUMMARY AND CONCLUSIONS**

1. A technic for the pre-excisional fixation of cancer in rats is described. Fixation *in situ* is most controllable when the fixative is incorporated in a plastic base.

2. Of many fixative chemicals tested, zinc chloride proved to be the most satisfactory, and its incorporation in bases containing a combination of permeant and agglutinant materials resulted in preparations of low general toxicity, capable of accurate control over a wide range of depths of fixation.

3. The histology of tissues fixed *in situ* is modified in various ways by different fixatives. Zinc chloride causes slight shrinkage of cytoplasm and nucleus but the tissue loses none of its diagnostic value. Thus the progress of the removal of a neoplasm can be controlled microscopically, assuring complete extirpation with a minimum of normal tissue destruction.

4. Treatment of cancer in rats by fixative chemicals has no tendency to increase metastasis even when this is purposely attempted by prolonged subcurative dosage.

**REFERENCES**

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