Observations on the Brown-Pearce Carcinoma in Roller Tube Tissue Cultures

G. O. Favorite, M. D., and F. S. Cheever, M. D.

(From the Department of Bacteriology and Immunology, Harvard University Medical School, Boston, Mass.)

(Received for publication November 16, 1940)

The present communication deals with observations made on the Brown-Pearce carcinoma in roller tube tissue cultures. This carcinoma, a highly malignant epithelial tumor discovered in the testicle of a syphilitic rabbit in 1920 (3), is readily transplantable from rabbit to rabbit by several routes, the intratesticular one being the method of choice. So far as is known to the authors, there is but one report in the literature concerning its cultivation in vitro.

In 1938 von Möllendorf (25) reported the successful propagation of the Brown-Pearce carcinoma over a 3 months' period employing the hanging drop and flask technic. The nutritive medium consisted of rabbit plasma and splenic extract, or in place of the latter testicle and lymph gland extracts. At first the addition of normal fibroblasts appeared necessary for the growth of tumor; in later subcultures it was possible to omit this step. After 3 months in vitro material injected into the testicle of a normal rabbit gave rise to the development of a typical testicular tumor which, in turn, was again propagated in tissue culture. In vitro 2 main types of cells were observed—round cells and spindle-shaped cells resembling fibroblasts. von Möllendorf believed that both types had a common origin and were involved in the development of the tumor; further, that round cells could become spindle-shaped cells, the former representing an exhausted stage and the latter a more active one. Morphologically she classified the tumor as a sarcoma and stressed the difficulty in distinguishing between sarcoma cells and normal connective tissue cells.

The roller tube method of tissue culture, originally suggested by Carrel in 1913 (4), first employed by Löwenstett (17) in 1925, and subsequently modified by Gey (9), Gey and Gey (10), and Lewis (16), has been successfully employed for the culture of normal tissues (9, 17), for the propagation of viruses (7, 11), and for the cultivation of malignant growths in vitro (10, 16).

Materials and Methods

The fundamental principles of the roller tube method of tissue culture have been summarized by Lewis (16). Plasma is evenly distributed over the inner surface of a thin-walled test tube and in this area are embedded fragments of the materials to be studied. Nutrient fluid is next added and the tightly stoppered tube is rotated in the horizontal position at 37°C. At desired intervals both fluid and gas mixtures may be changed.

Tissue.—The strain of the Brown-Pearce carcinoma, originally obtained from the Rockefeller Institute through the kindness of Dr. J. B. Murphy, had been carried for over a year in this laboratory by rabbit testicle inoculation. Vigorously growing testicular tumors or young metastases showing a minimum of necrosis were secured under aseptic precautions; any necrotic or nonmalignant tissue was removed and the remaining material minced into fragments of the desired size. The tissue was kept moist with Simms' solution (28) during the process.

Plasma.—Adult hens or cocks were bled by cardiac puncture or by venipuncture of a wing vein under sterile precautions, and by the addition of 0.01 per cent heparin, plasma was obtained.

Embryonic extract.—Three 10-day chick embryos, after removal of the eyes, were finely minced in 10 cc. of Simms' solution, incubated for 20 minutes at 37°C., rapidly frozen and thawed twice, and centrifuged for 10 minutes at 2400 R.P.M. The resulting supernatant fluid was pipetted off and stored in the refrigerator. The extracts were not kept longer than 12 days; in the majority of cases they were employed within 8 days.

Testicular extract.—The testicle of a normal rabbit was freed of tunica propria and of fat, thoroughly minced in 10 cc. of Simms' solution, incubated for 20 minutes at 37°C., rapidly frozen and thawed twice, and centrifuged at 2400 R.P.M. for 10 minutes. The supernatant fluid was pipetted off and stored in the refrigerator.

Splenic extract.—Splenic extract was made in a similar fashion except that 20 cc. of Simms' solution were used for each spleen.

Serum.—Adult normal rabbits were bled from the heart, the blood defibrinated, and clear serum obtained by centrifugation at 2400 R.P.M. for 20 minutes. This was stored in the refrigerator.

Simms' solution.—This solution was prepared essentially according to the method outlined by Sanders (28).

All materials were prepared with aseptic precautions and bacterial sterility was checked before they were used for the actual tissue culture work.

Assembly and incubation of culture.—Clean, sterile 20 x 150 mm. Pyrex test tubes were employed. Five
Brown-Pearce Carcinoma Cultures

OBSERVATIONS ON THE TUMOR EXPLANT IN VITRO

The cellular reaction which must be considered in interpreting the behavior of any tumor in vitro has been emphasized by several workers (6, 14, 15, 20, 29). If the explant is obtained from the testicle, one must consider, in addition to the tumor cells, the structures of the seminiferous tubules (spermatogenic cells and those of Sertoli), the interstitial cells and the connective tissue framework, and finally, mononuclear cells. Since the Brown-Pearce carcinoma is characterized in vivo by rapid growth associated with early and extensive necrosis, one must choose a portion of the tumor which appears to be in a relatively good state of viability. An area which contains many healthy tumor cells will have a variable amount of connective tissue stroma in addition to segments of well-preserved normal tissue mentioned above. It is evident, too, that besides the mononuclear reaction which accompanies the neoplastic invasion, there is an additional and similar one which is associated with the presence of necrotic tissue. As an aid in the recognition of the several types of cells, normal testicular tissue was often placed in the same tube with the tumor but at some distance from it. The use of metastases as a source of explants simplifies the problem somewhat, but here, too, necrosis is an outstanding feature and presents the same complications.

Various combinations of tissue extracts and rabbit serum were tried, including chick embryo, rabbit embryo, rabbit testicle, rabbit spleen and, simplest of all, plain serum in Simms' solution. Twenty-five per cent serum in Simms' solution was capable of maintaining the explants, but the addition of testicular or splenic extract enhanced the growth appreciably. The former proved to be somewhat the better of the two, but, regardless of the medium used, the behavior of the explants varied quantitatively rather than qualitatively.

Within 24 hours the fresh explants invariably presented a narrow zone of mononuclear cells. Occasionally the cells were small enough to be considered lymphocytes, but as a rule monocytes and macrophages made up the predominant cell type. Within 48 hours “strands” of fibroblasts began to appear from the margin of the culture. At the same time, the monocytes became more numerous, larger, irregular in size, and slightly granular. During the next few days, the comparatively wide zone of monocytes was gradually replaced by a fibroblastic outgrowth. The fibroblasts were usually of the long, spindle-shaped type, but the flattened, irregular, stellate, and multipolar varieties were quite frequent. In approximately 3 weeks the
fibroblasts formed the basic framework of the migrating corona of new tissue, enmeshing a variety of other cells, among which mononuclears predominated. Control cultures of normal testicle presented the same picture save for the presence of fewer monocytes.

The center of the explant always remained opaque at first. Its margin blended with the fibroblastic zone and from it could be seen emerging round cells believed to be tumor cells for the most part. At times, it was difficult if not impossible to distinguish tumor cells from those of the mononuclear type, particularly in young cultures. Usually about the 10th day (the time varying considerably), the surface of the explants would slough, revealing solid sheets of tumor cells beneath.

As viewed by transmitted light through the curvature of the culture tube (magnification: approximately 130 ×), the tumor cell was round, uniform in size, and slightly refractile. It was invariably in apposition with others, and its shape might assume a slightly oval or elongated form thereby, because of mutual pressure. No distinction could be made between the nucleus and cytoplasm because of the small amount of the latter present in young cultures. The cell shape appeared to be permanent. No transitional forms between a round cell and a fibroblastic type were ever seen. At the periphery of explants consisting essentially of tumor cells, oval and slightly elongated forms were occasionally noted. Here the cells at times assumed the sheet-like formation so common in cultures of epithelial tumors. The frieze of tumor cells proceeding from within outward consisted of round, oval, and finally flattened cells at the margin. In addition, a few fibroblasts were usually observed. In comparison to the densely packed cells of the central area, those at the periphery appeared to be separated from each other. With time, this became more marked, apparently being due to an increase in the amount of cytoplasm.

In hematoxylin-eosin preparations, the tumor cells did not differ much from those seen in the carcinoma proliferating in vivo. The cell was hyperchromatic; the nucleus was relatively large, vesicular, and presented a prominent nucleolus (Fig. 3). In older cultures, the cytoplasm tended to become granular (Fig. 4). The cell outlines were not always distinct, and in some areas presented a syncytial appearance. Mitotic figures were extremely rare in direct antithesis to the situation in the tumor proliferating in vivo.

A few cultures were treated with trypan blue by adding 1 drop of a 0.5 per cent solution of the dye in Simms' solution to 1.5 cc. of the nutrient fluid, which was renewed every other day. At the end of 72 hours, the dye was seen within the mononuclear cells and by the 5th day, bluish granules were noted in some fibroblasts when viewed through a blue filter. No tumor cells were observed to take up the dye.

Too frequent patching was quite detrimental to the tumor cells. Transplantation of explants or large fragments thereof invariably carried with it plasma clot. In many instances, the cells never left the old clot when transferred to their new environment. In such cases, the cells invariably perished. For this reason, transfer of explants was avoided when possible. The majority of our older cultures were maintained in the original tubes throughout their life span.

Frequently, after several weeks' growth, explants would be found to consist mainly of fibroblastic tissue. To circumvent this, 4 to 5 explants were placed in each culture tube. Invariably some of the explants would contain large masses of tumor cells. Again, it should be stressed that explants should contain neither too much necrotic debris nor, on the other hand, too much apparently healthy tumor, which may turn out to consist mainly of fibroblastic cells.

Isolation of the tumor cells in pure colonies could be accomplished in several ways. After a varying time, many of the extraneous cells would migrate from the explant, leaving behind a center of closely packed tumor cells. With sloughing off of dead material there would be left sheets of pure tumor cells (Fig. 2). Because of the rapid liquefaction of the clot by these cells, patching was often necessary. Unless this were done, large portions of the culture would disintegrate in the fluid medium or, as often happened, the cell masses would become attached to some other portion of the roller tube where they could be held in position by patching. Similar nests could be separated intentionally from the original explant by gentle teasing with a thin Pasteur pipette and then transplanted to another tube or to a fresh spot in the original one.

When tumor cells were isolated in pure culture they were quite inactive. The colony varied little in appearance from day to day (Fig. 1). The cells at the periphery might spread out slightly but it was difficult to say whether this was actual migration or merely passive movement due to the mechanical rotation of the culture tube. When well nourished, the cells remained clear, but when conditions were unfavorable they became small, shrunken, and pyknotic.

Recognition of the unstained tumor cell as it appeared in the orginal explants within the culture tube presented many difficulties. It soon became apparent that the cells of the advancing zone consisted of several types. von Möllendorf's observations in flask and slide cultures led her to believe that the tumor cells might assume round and fibrocytic forms. Since these were present in the authors' cultures also, it was originally assumed that both types might represent tumor cells. After several months' observations on over 100
Fig. 1.—A colony composed entirely of tumor cells, S. S. three days old. Cells show little tendency to migrate. In such colonies the cells appear merely to exist, with a certain number constantly showing degenerative changes. Unstained. X 140.

Fig. 2.—Liquefaction of the periphery of a colony, showing nests of tumor cells which can be transplanted to other portions of the same tube or to a new tube. Unstained. X 140.

Fig. 3.—Culture, 27 days old, composed entirely of tumor cells. Nucleoli are quite prominent, while the cytoplasm is minimal. Stained. H. and E. X 600.

Fig. 4.—Culture of tumor, 64 days old, showing the arrangement of the cells. The granular appearance of the cytoplasm is partly due to the heavy plasma coagulum. Stained. H. and E. X 600.
cultures, it gradually became evident that a large proportion of the cells in our cultures were not neoplastic. By systematic comparison of cultures of normal testicular tissue with those of the tumor itself, it was possible to form a definite opinion on the matter. The cultures of normal testicular tissue presented an almost identical picture at the marginal zone. The predominant cell was the fibroblast, usually of the broad, flat, irregular, and multipolar variety, although the fine fusiform type was also represented. A moderate number of mononuclear cells was always present. The fibroblasts obviously arose from the connective tissue framework of the testicle, which was quite abundant. It was not possible to recognize either the interstitial or Sertoli cells, as Mendelsohn reported (24). Single seminiferous tubules were isolated, and while the cells remained in a viable state and the connective tissue wall underwent proliferative changes, the development of spermatogenic cells was not observed. In tissue sections of 10-day cultures, these cells were the first to show necrosis, while those of connective tissue origin remained in good condition. It was essential to distinguish the former structures from tumor cells, particularly since spermatogenic cells in vivo contained numerous mitotic figures which conceivably might develop in vitro also and be mistaken for tumor cells. When these spermatogenic cells were liberated in the cultures through trauma or disintegration, they showed a characteristic appearance, since they were much smaller, with considerable variation in actual size from cell to cell.

Young, small mononuclears gave rise to much confusion. At times, it was impossible to distinguish them from tumor cells. In general, the latter retained their uniformity in size and shape and were invariably associated with similar cells in the form of masses or sheets. On the other hand, the mononuclear cells were usually larger, often migrated from one spot to another, usually increasing in size and becoming irregular in outline during the process. Furthermore, the nuclei were relatively small in comparison to the cytoplasm. As the latter became granular, it made a stronger contrast to the clear nuclei.

The ability of the 2 types of cells; i.e., fibroblastic and round cells, to produce malignant growths upon injection into susceptible animals will be considered in the next section.

Biological Tests of Tissue Culture Material

Tumor material cultivated for varying periods of time was injected in the form of saline emulsions into normal rabbits by the intratesticular route. In the first group, no effort was made to separate tumor cells from fibroblasts or round cells belonging to the monocyte-macrophage series. As was noted above, in spite of variations in the age of the cultures and in the type of fluid medium fed to them, in general a similar morphological picture was shown by all the cell nests. Of the 17 rabbits injected with these cultures, 10 or 59 per cent developed testicular tumors recognized clinically and proven at autopsy. A typical result is outlined in the following diagram, while the details are summarized below it.

A. Successful transfers:

<table>
<thead>
<tr>
<th>Number of culture</th>
<th>Age of culture in days</th>
<th>Number of days after inoculation when tumor was clinically recognized</th>
</tr>
</thead>
<tbody>
<tr>
<td>492 - 12</td>
<td>17</td>
<td>49</td>
</tr>
<tr>
<td>444 - 2</td>
<td>44</td>
<td>10</td>
</tr>
<tr>
<td>446 - 1</td>
<td>42</td>
<td>13</td>
</tr>
<tr>
<td>456 - 7</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>492 - 1</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>492 - 11</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>492 - 14</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>493 - 1</td>
<td>28</td>
<td>15</td>
</tr>
<tr>
<td>493 - 2 A</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>456 - 10</td>
<td>33</td>
<td>16</td>
</tr>
</tbody>
</table>

B. Unsuccessful transfers:

<table>
<thead>
<tr>
<th>Number of culture</th>
<th>Age of culture in days</th>
<th>Number of days after injection when tumor was clinically recognized</th>
</tr>
</thead>
<tbody>
<tr>
<td>202 - 1</td>
<td>24</td>
<td>40</td>
</tr>
<tr>
<td>432 - 1</td>
<td>62</td>
<td>21</td>
</tr>
<tr>
<td>432 - 6</td>
<td>50</td>
<td>54</td>
</tr>
<tr>
<td>456 - 8</td>
<td>10</td>
<td>64</td>
</tr>
<tr>
<td>456 - 15</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>492 - 16</td>
<td>17</td>
<td>52</td>
</tr>
<tr>
<td>493 - 2 B</td>
<td>14</td>
<td>4</td>
</tr>
</tbody>
</table>

* Animal died of intercurrent infection.
isted that some of them were members of the monocyte-macrophage series. Such cell nests were carefully removed and, after being ground in saline, were injected intratesticularly into normal rabbits. Positive results were obtained with 27 and 38-day old round cell cultures. Five other round cell cultures and 2 fibroblastic cultures gave negative results. The premature death of 4 animals from intercurrent infection may have reduced the number of successful inoculations. The results are summarized as follows:

<table>
<thead>
<tr>
<th>Number of Culture</th>
<th>Type of culture</th>
<th>Age of culture</th>
<th>Result</th>
<th>Number of days between injection and autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>432--10 A</td>
<td>Fibroblastic</td>
<td>38 days</td>
<td>No take</td>
<td>64</td>
</tr>
<tr>
<td>432--10 B</td>
<td>Fibroblastic</td>
<td>38 days</td>
<td>No take</td>
<td>64</td>
</tr>
<tr>
<td>432--10 C</td>
<td>Round cell</td>
<td>38 days</td>
<td>Take recognizable clinically</td>
<td>21</td>
</tr>
<tr>
<td>432-- 1</td>
<td>Round cell</td>
<td>27 days</td>
<td>Take recognized clinically</td>
<td>21</td>
</tr>
<tr>
<td>432-- 2</td>
<td>Round cell</td>
<td>31 days</td>
<td>No take</td>
<td>14 *</td>
</tr>
<tr>
<td>432-- 4</td>
<td>Round cell</td>
<td>22 days</td>
<td>No take</td>
<td>26 *</td>
</tr>
<tr>
<td>432--11</td>
<td>Round cell</td>
<td>41 days</td>
<td>No take</td>
<td>8 *</td>
</tr>
<tr>
<td>432--10 D</td>
<td>Round cell</td>
<td>38 days</td>
<td>No take</td>
<td>6 *</td>
</tr>
</tbody>
</table>

* Animal died of intercurrent infection.

IMMUNE BODIES

Since Carrel and Ingebrigtsen (5) demonstrated the production of antibodies by tissues proliferating in vitro, attempts to investigate the nature of immunity to certain transplantable tumors by this method have been complicated by the difficulty in obtaining an antiseraum simultaneously effective against malignant cells and innocuous to normal cells of the host. By injecting rats with suspensions of mouse carcinoma M63, Lumsden and his associates (13, 21-23) have reported the production of a serum toxic to the malignant cells in vitro, while neighboring colonies of normal cells in the same tube remained unharmed. Neither Phelps (27) nor Ludford (18, 19) was able to confirm this work, and the latter has criticized it on the basis that Lumsden did not distinguish between normal and malignant cells in his tissue cultures. As it was relatively easy to procure rabbits rendered refractory to inoculations of the Brown-Pearce carcinoma, either by the intracutaneous technic first used by Brown and Pearce and later by Besredka (1, 2) or by selection of those rabbits in which testicular implantations had naturally regressed, it seemed worth while to repeat these experiments, using sera and tissue extracts from these "immune" rabbits. Furthermore, in line with Kidd's observation (12) of a complement binding substance associated with this tumor, attempts were made to demonstrate this same factor in vitro.

So far as the experiments went, there was no conclusive biological evidence to suggest the existence of humoral or tissue antibodies effective against tumor cells in vitro. Because tumor cells in pure culture lacked vigor and proliferative ability, it was necessary to use fresh explants, as suggested by Santesson (30). The effect of various combinations of serum from normal and immune rabbits plus splenic and testicular extracts from normal and immune rabbits was investigated. Three series of cultures, with proper controls, were set up and fed the immune or normal nutritive fluid for at least 10 days. No significant differences in the rate of growth were noted by direct observation and measurements, but it was difficult to evaluate the results, since the natural outgrowth of the explants consisted mainly of fibroblasts and mononuclear cells, which, as were expected, showed no ill effect when fed immune serum or tissue extracts. The centrally located, closely packed tumor cells did not permit accurate observations but, as far as could be seen, there was no change in their microscopic appearance to suggest that any of the media fed them had an inhibitory effect. When fragments of spleen or testicle from an immune animal were growing in the same tube with the tumor, but at some distance from it, the subsequent course of the tumor was not altered.

Nine cultures of apparently typical tumor tissue fed normal serum plus, in some cases, normal tissue extracts were injected into rabbits by the intratesticular route. Four, or 44 per cent, developed typical takes. Of 7 fragments exposed in some form to serum or tissue of immune animals, 3, or 43 per cent, gave positive results upon intratesticular injection. These 7 fragments were treated as follows:

Positive Results:

1. Fed immune serum plus Simms' solution daily for 17 days. Tumor recognizable clinically 13 days after injection.
2. Fed immune serum plus Simms' solution plus immune splenic extract daily for 17 days. Tumor recognizable clinically 13 days after injection.
3. Fed normal serum plus Simms' solution daily for 10 days. Testicular tissue from immune rabbit growing in same tube. Tumor recognizable clinically 13 days after injection.
Negative Results:

a. Fed normal serum plus Simms' solution plus immune testicular extract daily for 50 days. No take.
b. Fed immune serum plus Simms' solution daily for 10 days. Testicular tissue from immune rabbit growing in same tube. No tumor.
c. Fed immune serum plus Simms' solution plus immune testicular extract daily for 17 days. No tumor.
d. Fed immune serum plus Simms' solution daily for 17 days. Splenic tissue from an immune rabbit growing in the same tube. No tumor.

COMPLEMENT FIXATION

A serologically active principle associated with saline extracts of the Brown-Pearce carcinoma occurring in vivo has been reported by Kidd (12) to fix complement in the presence of serums obtained from rabbits bearing the specific tumor or from those in whom the growth has recently regressed. Attempts to demonstrate a similar substance from tumor grown by the roller tube tissue culture technic have met with failure. Fragments of tissue from 18 tubes (the age varying from 10 to 44 days) were carefully removed, ground up, and extracted in saline according to Kidd's technic, which was followed throughout. Six extracts proved anticomplementary and were discarded. The remaining 12 did not fix complement with any of the serums, normal or "tumor bearing," which were tried. Nutritive fluid (Simms' solution plus normal serum) in contact with the tumor cells for 48 hours gave negative results. As a last resort sterile complement and sterile serum from tumor-bearing or normal rabbits were added to the tissue culture tubes which were replaced in the incubator to be rotated for 1 or 2 hours before samples were withdrawn and added to the hemolytic system in order to test for the presence of free complement. In every case, however, complement had been destroyed apparently by the combined effect of the deteriorating temperature, 37°C, in the incubator and the anticomplementary quality of the tissue culture.

In theory it is possible that had there been more tumor material available from the roller tubes enabling one to make a more concentrated extract, fixation of complement might have been demonstrated.

DISCUSSION

The roller tube method of tissue culture appears to furnish a technic whereby the cells of the Brown-Pearce carcinoma may be maintained for long periods of time. Direct observations carried out daily over a period of 5 months and study of the numerous photographs taken suggested that the tumor cell retained its characteristic form in vitro. It was felt that it could be distinguished from cells of inflammatory origin in all but young cultures. It furthermore retained its malignant characteristics, since it reproduced typical growths when injected into the testicles of normal animals after varying periods of cultivation. The longest one was 44 days in vitro. On several occasions it was possible to isolate cell nests consisting of pure tumor cells as far as could be determined. Upon injection of these cell masses into susceptible animals typical growths were produced in 2 out of 6 cases.

Most, if not all, of the outgrowth of tissue explants was the result of sustained proliferation of fibroblasts and mononuclear cells which made up the connective tissue stroma of the tumor. The carcinoma cells themselves remained massed in the central part of the explant, showed very little tendency to migrate, and since but 2 mitotic figures were seen in the course of 5 months' observations, one could not be certain that the malignant cells were actually reproducing themselves instead of merely persisting within the tissue explant. In connection with this, it may be remembered that von Möllendorf observed 2 types of tumor cells in her cultures of the Brown-Pearce carcinoma; viz., a round cell type and a spindle-shaped fibroblastic one. On morphological and biological criteria the present authors believe that the spindle-shaped fibrocytic cells represented true fibroblasts arising from the connective tissue stroma of the tumor and that carcinoma cells in vitro resembled those in vivo, retaining their typical round cell morphology. Careful study was required in order to distinguish them from round cells of the monocyte-macrophage series.

As Fischer (8) has pointed out, some tumor cells need a framework for their growth. This appeared to be true for the Brown-Pearce carcinoma. In pure cultures, the cells showed little activity, seeming merely to exist (Fig. 1). Although they appeared to do this well, it was apparent that the mortality rate among them was high enough to insure the death of the colony in the course of time, unless multiplication occurred. Just how long these pure cultures would survive is impossible to say since at the termination of our experiments the cells were still viable as judged by their general appearance.

Immunological studies were admittedly inconclusive, but in the few experiments recorded there was no definite evidence to suggest that serum or tissue extracts from immune animals had any inhibitory effect upon the growth or malignant characteristics of the tumor cell cultivated in vitro.

During the course of these experiments certain shortcomings of this method of tissue culture were noted.

Rigid adherence to a routine technic and a strict observance of precautions designed to insure asepsis did not prevent a high percentage of contaminations. The use of fresh extracts and serums necessitated frequent preparations of these ingredients. This, coupled with daily changes of the fluid in the culture tubes, appeared to be the chief source of contamination.

Downloaded from cancerres.aacjournals.org on November 3, 2021. © 1941 American Association for Cancer Research.
The inability to use a higher magnification in the study of cellular details was due to the curvature and thickness of the wall of the culture tube. For the same reason, it was impossible to use dark field illumination.

All of the cultures fixed in situ with 10 per cent formalin were easily stripped off along with the plasma clot which covered the surface of the tube, and were for the most part stained directly after dehydration in alcohol. The young cultures were satisfactory, but the older ones became enmeshed in a relatively heavy coating of clotted plasma unless they had been transplanted several times. This coating interfered quite seriously with the study of cellular detail. A few fragments were removed in toto and then embedded and sectioned, but these too were generally unsatisfactory because of the thinness of the original pieces of tissue.

**Summary**

1. The roller tube culture technic appeared to furnish a practicable method for the maintenance in vitro of the specific cells of the Brown-Pearce carcinoma.

2. After 44 days in tissue culture the carcinoma cells still retained their ability to produce malignant growths in susceptible animals.

3. Definite evidence that these carcinoma cells proliferated in vitro was not obtained.

4. The round tumor cells retained their characteristic morphology throughout the course of the experiments and no transition to a spindle-shaped fibroblast was observed.

5. Serums and tissue extracts from animals immunized against the tumor showed no definite capacity to exert a cytotoxic action on the tumor cells in vitro.

We wish to express our appreciation of the aid and advice generously given by Dr. A. M. Brues of the Huntington Memorial Hospital.

**References**


Observations on the Brown-Pearce Carcinoma in Roller Tube Tissue Cultures

G. O. Favorite and F. S. Cheever


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/1/2/136.citation

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/1/2/136.citation. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.