Lytic Action of Candida and Saccharomyces Sp. on Sarcoma 37 and Ehrlich Ascites Tumors

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In 1946 Protti (8) described the lytic action of several species of Saccharomyces on tumor cells in vitro by a process which he called "cytophotolysis." This was studied further by Castelli and Gaggini (1), who reported that not only common yeasts but also Oidium (i.e., Candida albicans) lysed tumor cells when incubated for 24 hours at 37° C. in glucose solution, although dissociated normal liver cells under the same circumstances were not affected; and that the development of transplantable adenocarcinoma could be suppressed by injecting host mice with 0.2 cc. of a 1:16 suspension of living C. albicans at 2-day intervals. According to these authors, no tumors developed up to 45 days, and at that time the grafts were shown to contain pus or were hemorrhagic and degenerative; furthermore, if treatment was delayed until 10 days after transplantation, tumors developed, but never grew to a large size.

In a series of studies (5—7) Lewisohn et al. investigated the effects of yeasts on growth and regression of transplantable mouse Sarcoma 180 and spontaneous mammary carcinoma. Their initial experiments involved the regression of 50 per cent of the spontaneous tumors in mice fed a normal diet and in 60 per cent of those fed on a polished rice diet after 4—6 weeks of daily intravenous injections of yeast extract. Sugiura (10) was unable to repeat the experiments with an extract prepared by Merck and Co. In 1951 Gottschalk (3) reported beneficial effects and partial regression of a few tumors following in vivo treatment of human cancer patients with intravenous injections of living yeasts.

In our experiments, fluid from 3-day implants of ascites tumors (Sarcoma 37 and Ehrlich) was incubated with suspensions of yeasts or yeast-like organisms in 4.7 per cent glucose solution and was able to substantiate the findings of these authors with respect to lysis of tumor cells, but was not convinced that there was not some effect, though slower and less drastic, on the liver cells as well.

In our hands some of the control liver cells were always damaged by the experimental procedure, described below, even before the suspensions of the organisms were added. Liver preparations were therefore not considered by us to be an adequate control for ascites tumor cells, which suffered no damage in preparation. Furthermore, liver cells are completely differentiated and in this respect also are not good controls for rapidly dividing, relatively undifferentiated tumor cells. We therefore repeated our experiments in tissue culture, using embryonic heart preparations as controls.

MATERIALS AND METHODS

In our experiments, fluid from 3-day implants of ascites tumors (Sarcoma 37 and Ehrlich) was incubated with suspensions of yeasts or yeast-like organisms in 4.7 per cent glucose solution. Normal mouse liver tissue from 6-week-old Swiss mice was used as a control. The organisms to be tested were washed with sterile saline and centrifuged at 2,000 r.p.m. in a tared centrifuge tube. The supernatant was pipetted off, the weight of the centrifugate calculated, and the sediments cell suspension reconstituted to a volume of approximately 10 mg/cc of glucose solution. Liver tissue was finely minced and further dissociated by force-
ing through a 20-gauge needle. To 1 cc. of the ascites fluid or liver preparation, respectively, an equal amount of the yeast suspension was added, the mixture incubated at 37° C., and samples studied with the phase microscope at 24-hour intervals. Fixed preparations were stained with Kligman's modification of the Hotchkiss-McManus technic for study of fungi and counterstained with Harris' hematoxylin (Fig. 1).

Tissue cultures were prepared according to Sano's cover slip technic (9), with the exception that we used plastic instead of glass rings. Four 1-c. mm. explants were placed in each chamber in 3 drops of Tyrode's solution. Three drops of human cord serum, 3 drops of chick embryo extract, and 3 drops of Difeo chicken plasma were added, and the chamber was closed with a vaseline-paraffin seal. The cultures were allowed to stand until the clot was complete and then incubated at 37° C. Forty-eight hours later, 1 drop of a volumetric dilution of 1:10,000 yeast cells was added to each culture. For phase contrast observation or photography at desired intervals, the cover slip chambers were inverted and placed on the stage of the microscope. Stained preparations were made by removing the cover slip to which the clot was attached and passing it through the necessary solutions without disturbing the explants.

Tumor preparations were made in essentially the same way. Sarcoma 37 ascites fluid was removed aseptically from a mouse and diluted 1:1 with 4.7 per cent glucose. One drop of this suspension was added to each culture chamber, and incubated, with or without yeast suspensions prepared as above.

In vivo studies were made on Swiss mice implanted with Sarcoma 37 ascites fluid. Into each mouse, yeast cells suspended in saline solution (10 mg/cc) were injected intraperitoneally, 72 hours after implantation of the tumor. McManus-Hotchkiss-stained smear preparations were made of the fluid withdrawn at 24-hr. intervals thereafter, and the survival time of infected and control animals noted.

OBSERVATIONS

In Vitro

Glucose preparations.—Table 1 shows the results obtained by incubating ascites tumor cells (Sarcoma 37 and Ehrlich) in glucose suspension with the following species of Candida: C. albicans, C. stellatooides, C. tropicalis, C. pseudotropicalis, C. Krusei, C. parakrusei, and C. Guillermondii. Rho- dotorula rubrum and several cultures of Saccharomyces were also tested. Arbitrary degrees of lysis have been indicated, depending on the rapidity of action and the degree of degeneration over a given period. The organisms showing the greatest oncolytic activity with respect to Sarcoma 37 in vitro were the various stocks of Saccharomyces (Fig. 3), the "Paris 97" strain of C. albicans isolated from a Hodgkin's patient (Fig. 4), and C. Krusei (Fig. 2). Other cultures—e.g., Candida albicans (strain 57) and C. parakrusei—had a strongly lytic effect on Ehrlich ascites cells under the same conditions, but much less effect on Sarcoma 37.

Organisms capable of producing lysis usually were found adhering to cell membranes by 24 hours or within the cytoplasm of the tumor cells (Fig. 6). Cell destruction does not necessarily depend on intracellularity, however, as is shown in the case of C. Krusei, which caused degeneration of Sarcoma 37 cells in vitro inside 48 hours, without coming into contact.

Though control ascites cells ceased to divide, they did not appear degenerative during the first 48 hours. However, at 72 hours some fatty degeneration of the control cells (extrusion of fat, vacuolization) had begun, and the preserved cells exhibited cloudy staining.

Tissue culture.—The technic used in study of Sarcoma 37 ascites and control cells in tissue culture has been indicated under "Materials and Methods." At 24 hours after infection of a Sarcoma 37 culture with Candida albicans, rounding up of some tumor cells was noted. By 48 hours yeasts were found inside the cells, which showed pyknosis, crenulation, nuclear damage, or total destruction (Figs. 8, 9). The control sarcoma cells continued to proliferate (Fig. 7) throughout the experiment. The same was true of the control heart tissue (Fig. 10), whether or not it was infected with C. albicans. Figure 11 shows a fibroblast culture 48 hours after infection. Although mycelium has overgrown the culture chamber and some organisms are in close proximity to the cells, there is no evidence of retraction of cell processes or of any cellular damage due to the organism (Fig. 12). This was true in all cases where rapidly proliferating 3-day explants were used as starting material. In older tissue cultures in which cells had begun to degenerate, with piling up or extrusion of fat droplets, we sometimes obtained a different picture. In such cultures intracellular organisms were found frequently.

According to Castelli and Gaggini, differences in response of normal cells and tumor cells to C. albicans are apparently not the consequence of differences in pH. In our experiments, the pH (Beckman determination) of undiluted Sarcoma 37 ascites fluid, immediately after withdrawal
from the peritoneum, was 7.2. Readings ranging from pH 6.2 to 5.6 were obtained in different preparations after dilution with glucose solution, but no further drop was experienced on addition of the yeast suspension. All our tissue cultures, on addition of Tyrode's solution, gave initial readings of pH 7.7–7.8. During the first 48 hours of outgrowth, the explants became less alkaline (normal cell preparations, pH 7.2–7.3; ascites cell preparations, pH 6.4–6.8). The pH of both infected and uninfected tumor preparations continued to drop during the 48–96-hour period; but since no lowering of pH was exhibited by either control or Candida-infected normal cell preparations during the same period (final pH, 7.3 ±.02), these changes cannot be ascribed to the action of the infective organisms. It could, perhaps, be thought that the organisms have a more destructive effect in an acid environment. However, this does not seem to be borne out by the glucose isolation experiments, where the range of values for both liver cell and ascites cell preparations, as mentioned above, was pH 6.2–5.6, and where the differentially destructive action of the organisms on tumor cells is most strikingly demonstrated.

In Vivo

Swiss mice from our colony stock were implanted with Sarcoma 37 ascites, and the tumor was allowed to develop for 3 days until visible ab-

TABLE 1

ACTION OF ORGANISMS ON SARCOMA 37 AND EHRLICH ASCITES IN VITRO
(Incubated in isotonic glucose)

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>TUMOR</th>
<th>24 hrs.</th>
<th>Cellular Changes in Tumors</th>
<th>48 hrs.</th>
<th>72 hrs.</th>
<th>Degree of Lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans Strain #57</td>
<td>S-37 ascites</td>
<td>yeasts adhering to cell membrane</td>
<td>staining reaction</td>
<td>faint total lysis</td>
<td>lytic of nuclear structure</td>
<td>0</td>
</tr>
<tr>
<td>* Strain Paris 97</td>
<td>S-37 ascites</td>
<td>cells degenerating organisms and cells fragmented</td>
<td></td>
<td></td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td>Candida Guillermonti</td>
<td>S-37 ascites</td>
<td>*</td>
<td>cellular degeneration without contact</td>
<td></td>
<td>McManus-positive inclusion bodies</td>
<td>0</td>
</tr>
<tr>
<td>Candida Krusei</td>
<td>S-37 ascites</td>
<td>*</td>
<td>cellular degeneration without contact</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>* Ehrlich ascites</td>
<td>fatty degeneration</td>
<td>intracellular organisms; cells lysed</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Candida pseudotropicalis</td>
<td>S-37 ascites</td>
<td>degeneration of many cells damaged</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>* Ehrlich ascites</td>
<td>S-37 ascites</td>
<td>degeneration of many cells damaged</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Candida parakrusei</td>
<td>S-37 ascites</td>
<td>some cell damage complete lysis</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Candida stellatoideos</td>
<td>S-37 ascites</td>
<td>a few cells show intracellular organisms</td>
<td>15 per cent of cells damaged</td>
<td>complete lysis</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>S-37 ascites</td>
<td>Ehrlich ascites</td>
<td>same as C. Krusei cellar debris</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>S-37 ascites</td>
<td>organisms intracellular</td>
<td>cells degenerative debris only</td>
<td></td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td>Stock culture</td>
<td>S-37 ascites</td>
<td>organisms intracellular</td>
<td>cells degenerative debris only</td>
<td></td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td>* Wahl cultures</td>
<td>S-37 ascites</td>
<td>severe damage intracellular yeasts</td>
<td>lytic lytic lytic lytic</td>
<td></td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td>&quot;Alcoless&quot; (AW)</td>
<td>S-37 ascites</td>
<td>&quot;</td>
<td>lytic lytic lytic lytic</td>
<td></td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td>* (AWS) stock (RS)</td>
<td>S-37 ascites</td>
<td>&quot;</td>
<td>lytic lytic lytic lytic</td>
<td></td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td>Controls</td>
<td>S-37 ascites</td>
<td>continued mitosis normal some fatty degeneration</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Controls</td>
<td>Ehrlich ascites</td>
<td>continued mitosis normal</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

+ 25–50 per cent damaged.
+++ 50–75 per cent damaged.
++++ Over 75 per cent damaged.
DISCUSSION

Thus far, the mechanism whereby the lytic action is achieved has not been elucidated; but experiments to test the effect of different chemical fractions of the most active cultures are now being undertaken in collaboration with other members of our Institute.

Castelli and Gaggini could find no evidence that the results were due to toxicity of glucose, since the outcome was the same when tumor cells were isolated in physiological salt solution and incubated with C. albicans. We have maintained control tumor cells in 4.7 per cent glucose for as long as 7 days without marked cellular degeneration.

Several cultures of Saccharomyces, including a stock culture and three strains ("Alcoless" AW, "Alcoless" ASW and RS) kindly supplied us by Mr. Manfred Wahl, were also tested in vivo. The Wahl strains were pure cultures derived from single cell isolates, two of which produce very low percentages of alcohol, or none, in the presence of various sugars (11). No difference was noted in the effects of these different cultures. All of them induced inflammatory reactions in the peritoneal cavity of the host, and the living organisms were ingested by leukocytes. Survival time of all mice treated with Saccharomyces in vivo was the same as that of controls.

Although, as mentioned earlier, actual contact of the organisms with the tumor cells does not appear to be necessary for the production of lysis in vivo, nevertheless only intracytoplasmic organisms such as C. albicans (see Fig. 6) caused notable cellular damage in vivo.

and occasionally have found cells in mitosis at 66 hours, following an initial period of inhibition.

Protti (8), without attempting chemical analysis, considered the action to be an enzymatic process which he called "cytophotolysis." Lewi sohn et al. (5-7) reported that the active principle of their extracts was nonprotein in nature, was water-soluble, and that it could be precipitated by lead acetate and silver nitrate.

Whether or not the penetration of the tumor cell is achieved through action of the organism on the cell membrane or whether the tumor cell phagocytizes the foreign body is not clear. However, some species of Candida become intracellular during the first 24 hours, whereas cells of other species always remain outside the cell. If the phenomenon is due to phagocytic action of the tumor cell, it would be difficult to understand this selectiveness. In our stained preparations it was noted that organisms in contact with cell membranes...
were often surrounded by a halo at the border of which the cell appeared to be degenerating, and tumor cells with intracellular organisms frequently presented the appearance shown in Figures 4 and 5, where a ragged hiatus in the cell membrane may be observed.

**SUMMARY**

Sarcoma 37 ascites and Ehrlich ascites tumor cells were incubated in 4.7 per cent glucose at 37° C. in the presence of various yeasts or yeast-like organisms. In 24 hours the organisms were intracellular, and many cells were damaged or lysed; and by 48 hours the tumor cells were completely destroyed, although most of the normal mouse liver cells similarly treated appeared viable, and control tumor cells were still morphologically intact, though not dividing. Particularly effective was a culture ("Paris 97") of C. albicans isolated from a Hodgkin's patient, and several cultures of Saccharomyces. Similar results were obtained with C. parakeuei, C. stellatoideas, and C. tropicalia. C. Krusei, C. psuedotropicalis, and C. Guillermondi were also active, but to a lesser degree.

Sarcoma 37 cells grown in tissue culture with Candida albicans degenerated completely in 24—48 hours after infection, while normal fibroblasts from embryonic Swiss mice, cultured under the same conditions, were not affected.

Living Candidas or yeasts injected into the peritoneal cavity of mice bearing ascites tumors were quickly phagocytized by leukocytes and were only occasionally observed inside the tumor cell. Inhibition of mitosis for periods ranging from 24 to 48 hours and some reduction of ascites swelling followed in vivo intraperitoneal injection of the "Paris 97" culture of C. albicans, but the survival time was the same as that of control mice.

**REFERENCES**

Fig. 7.—Sarcoma 37 ascites cells grown in tissue culture, 48 hrs.

Fig. 8.—Preparation from same tumor grown in another chamber and infected with C. albicans (Paris 97 culture). No healthy cells remain at 48 hrs. post-infection.

Fig. 9.—Individual cells from same preparation as Figure 8, higher magnification.

Fig. 10.—Fibroblast culture from normal mouse embryo (control).

Fig. 11.—The same, 48 hours after infection with C. albicans. No destruction of cells detectable.

Fig. 12.—High power study of cells from same preparation as Figure 11. Cells appear completely healthy in contrast to tumor cells (Fig. 9).
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