The Cytotoxic Effects of Hemolytic Streptococci on Ascites Tumor Cells*

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SUMMARY

The effect of living hemolytic streptococci on ascites tumor cells (Sarcoma 37 and Krebs-2 carcinoma) was studied in vitro by phase microscopy and in vivo by implantation into mice. The effects produced by Streptococcus pyogenes strains were compared with those of Serratia marcescens, Sarcina lutea, and Streptococcus faecalis. Three of the eight streptococcal strains were highly effective against the tumor cells, resulting in reduced number of tumor "takes" and reduction of tumor sizes, whereas S. lutea, S. marcescens, and S. faecalis were only partially effective in damaging tumor cells after in vitro incubation. The cytological changes are recorded by phase photomicrography, and the histopathological findings of the tumor implantation sites are described.

In previous studies toxins from Serratia marcescens, other gram-negative bacteria (8, 5, 8) and the combined toxins from Streptococcus pyogenes and S. marcescens were found to cause regression of well established transplanted mouse tumors (Sarcoma 37 and Krebs-2 carcinoma). Streptococcal preparations were ineffective against these well established solid mouse tumors when used alone, but they enhanced the oncolytic effect of Serratia preparations and lowered their toxicity (5, 8). Koshimura et al. (10, 13, 14) found that living hemolytic streptococci were cytotoxic for Ehrlich ascites tumor cells. In an in vivo-in vitro experiment these authors incubated the ascites cells with a resting bacterial cell suspension and, on reimplantation of the treated cells, obtained inhibition of tumor "takes" in a significant number of mice as compared with the untreated controls. Incubation of the tumor cells with heated streptococci, streptococcal filtrates, or other microorganisms (pneumococci, enterococci, a-hemolytic streptococci) produced no visible cellular damage. No data on cytopathogenic changes were given by this group.

I. Ginsburg and N. Grossowicz (4) studied the effect of several streptococcal hemolysins on Ehrlich ascites tumor cells in vitro, using stainability with Trypan blue as the criterion for cytotoxic damage. Their results indicated that these hemolysins (Hemolysin O, SLD, and CBH) were toxic for the tumor cells as well as for normal mouse and rabbit leukocytes and chicken fibroblasts.

The capacity of streptococci to lyse red blood cells and leukocytes has long been recognized (1, 12, 15). Hemolytic streptococci have also been found to be cytotoxic for mouse ascites tumor cells in vitro (6, 7). The studies reported here were undertaken to determine whether the organisms used for bacterial toxin studies (5, 8) exert a direct cytopathogenic effect. The cellular damage produced by streptococci and other microorganisms on two types of ascites tumor cells responsive to toxin therapy, Sarcoma 37, and Krebs-2 carcinoma are described. These cellular changes were recorded by phase-photomicrography (6), and in addition the viability of the treated cells was tested by reimplantation into mice. The number of tumor "takes," tumor sizes, and the survival times were taken as criteria of tumor cell damage induced by the bacteria (6, 7).

MATERIALS AND METHODS

Preparation of bacterial suspensions.—The bacterial cultures, Streptococcus pyogenes D, E, S.A, Serratia marcescens, Sarcina lutea, and Streptococcus faecalis;4 were grown for 24 hours in brain damage. Their results indicated that these hemolysins (Hemolysin O, SLD, and CBH) were toxic for the tumor cells as well as for normal mouse and rabbit leukocytes and chicken fibroblasts.

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heart infusion broth (at 37°C for the Streptococci and at 25°C for Serratia and Sarcina), centrifuged, washed twice with chilled saline, and resuspended in Mammalian Ringer in one-twentieth of the original culture volume, thereby achieving a twentyfold concentration. Serratia marcescens cultures were resuspended in only one-half of their original volume (twofold concentration) because of their high toxicity.

**Mice.**—Male ICR albino mice, 8–10 weeks old and weighing 30–34 gm. were used for all experiments. Ascites donor mice were usually 10–13 weeks old and weighed 34–36 gm.

**Ascites tumor suspensions.**—Ascites cells were harvested from donors (male ICR albino mice) bearing 7-day-old ascites tumors. The tumor cells were centrifuged, washed twice with chilled saline, and resuspended in Mammalian Ringer to contain 80–90 million tumor cells per ml.

**Bacteria-tumor incubating mixture.**—The twice-washed tumor cells were incubated for 1½ hours with Mammalian Ringer and resting cell suspensions of bacteria. At the end of the incubation period penicillin or chloramphenicol (Chloromycetin) was added, and 0.5 ml. of the following incubated mixture was injected into the mice (see Tables 1 and 2).

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th><strong>Development of solid tumors following subcutaneous injection of bacteria-treated Sarcoma 37</strong></th>
<th><strong>Krebs-2 carcinoma</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Groups</strong></td>
<td><strong>Sarcoma 37</strong></td>
<td><strong>Krebs-2 carcinoma</strong></td>
</tr>
<tr>
<td></td>
<td><strong>No. mice</strong></td>
<td><strong>Tumor size av.</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Days</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>7 (Sq. cm.)</strong></td>
</tr>
<tr>
<td>1. Controls</td>
<td>80</td>
<td>2.3</td>
</tr>
<tr>
<td>2. Penicillin controls</td>
<td>40</td>
<td>2.1</td>
</tr>
<tr>
<td>3. Chloramphenicol controls</td>
<td>40</td>
<td>2.3</td>
</tr>
<tr>
<td>4. <em>Serratia marcescens</em></td>
<td>88</td>
<td>1.9</td>
</tr>
<tr>
<td>5. <em>Sarcina lutea</em></td>
<td>71</td>
<td>1.1</td>
</tr>
<tr>
<td>6. <em>Streptococcus faecalis</em></td>
<td>36</td>
<td>1.3</td>
</tr>
<tr>
<td>7. <em>Streptococcus pyogenes D</em></td>
<td>48</td>
<td>0.3</td>
</tr>
<tr>
<td>8. <em>Streptococcus pyogenes E</em></td>
<td>48</td>
<td>0.3</td>
</tr>
<tr>
<td>9. <em>Streptococcus pyogenes SA</em></td>
<td>47</td>
<td>1.4</td>
</tr>
<tr>
<td><strong>Total no. mice</strong></td>
<td>438</td>
<td></td>
</tr>
</tbody>
</table>

All mice were given inoculations subcutaneously in the intrascapular region of 0.5 ml. of incubated tumor cell suspensions of the following composition:

- **Group 1.** Controls: 0.1 ml. of tumor cell suspension (80–90 million cells) and 0.4 ml. of Mammalian Ringer.
- **Group 2.** Penicillin controls: 0.1 ml. of tumor cells; 0.3 ml. of Mammalian Ringer; 0.1 ml. of penicillin (10,000 units) or 0.1 ml. of chloramphenicol (250 μg.) added after 1½ hours' incubation period.
- **Group 3.** Chloramphenicol controls: 0.1 ml. of tumor cells; 0.3 ml. of Mammalian Ringer; 0.1 ml. of chloramphenicol (250 μg.) added after 1½ hours' incubation period.
- **Group 4–9.** Bacteria-treated tumor cells: 0.1 ml. of tumor cells, 0.2 ml. of resting cell suspension of bacteria, 0.1 ml. of Mammalian Ringer and 0.1 ml. of penicillin (10,000 units) or chloramphenicol (250 μg.) added after 1½ hours' incubation period.

**Viability test of bacteria-treated tumor cells.**—
The viability of the bacteria-treated tumor cells was tested by reimplantation of the incubated mixture subcutaneously or intraperitoneally into groups of sixteen mice. Survival, tumor size, and number of tumor "takes" were used as indices of tumor cell damage.

Persistence of microorganisms in hosts.—Experiments were terminated only 90–100 days after tumor implantation because of the occurrence of occasional late tumor growth following the injection of the bacteria-treated tumor cells. All mice were heart-bled and autopsied at the time of death or at the termination of each experiment. The animals were examined for tumor foci, and the blood was cultured to check the persistence of the induced infection (Table 3).

Phase microphotography.—A Zeiss Phase Microscope with built-in illuminator and a camera attachment was used (GLF 658–682), a phase-contrast condenser (III, Z/6 with long working distance), and two objectives: Achromat Phase 40/0.65 and Achromat Phase 100/1.25 oil. All pictures were taken with 35-mm. Kodak Plus-X Pan film. Tumor cell suspensions and bacteria-treated tumor cells were observed routinely before and after incubation under phase before each animal experiment to check the tumor cell damage and possible bacterial contaminants. Tumor-bacterial cell suspensions for phase photomicrography were prepared as previously described, and pictures were taken at stated time intervals (see legend) which refer to incubation time after the bacterial cell suspension was added to the tumor cells.

Histopathology.—Tissue changes were studied in five different groups of mice which were given injections as follows: Group I, Streplococcus pyogenes SA control; Group II, tumor cells incubated with Streplococcus pyogenes SA; Group III, Serratia marcescens control; Group IV, tumor cells incubated with Serratia marcescens; Group V, tumor cell control. Preparation of bacterial and tumor cell suspension was identical to that described in the preceding section except that groups I and III were given injections of bacterial suspensions without the addition of tumor cells. At the end of 1½ hours' incubation at 37°C, penicillin or chloramphenicol was added to all groups. Animals were sacrificed at 24, 48, 72 hours, 1-week, 2-week, and 3-week intervals after implantation. At autopsy the injection sites, lymph nodes, thymus, spleen, lungs, liver, adrenals, and sternum were taken for tissue studies.

RESULTS

Results obtained in vivo.—Streplococcus pyogenes strains severely damage and eventually kill ascites tumor cells after in vitro incubation,
whereas other bacterial strains have little or no effect on the tumor cells.

The cellular changes following incubation of tumor cells with the bacterial suspensions are depicted in Figures 1–11 and the in vivo tests are summarized in Tables 1 and 2. Results obtained by subcutaneous injection in the intrascapular region of bacteria-treated Sarcoma 37 and Krebs-2 carcinoma cells are presented in Table 1.

The first column lists the experimental groups and the bacterial strains used in this study. From the average tumor sizes on days 7 and 21 of both Sarcoma 37 and Krebs-2 carcinoma it can be seen that there was a considerable reduction in size by 21 days in the groups of mice having received streptococcus-treated tumor cells as compared with the controls. This was not so apparent at 7 days when tumors were just becoming established and some degree of inflammation existed in all groups. The survival of those animals showing tumor growth was not changed greatly by the bacterial treatment (see last column, Table 1, Part I). The number of “takes” is probably the most crucial test of the cytological damage inflicted by the bacteria. For Sarcoma 37 tumor “takes” in the controls were 100 per cent, but they were 0 per cent in the S. pyogenes SA-treated group, 27 per cent in the S. pyogenes D and 32 per cent in the S. pyogenes E-treated group; results fall between the treated and controls in all the other strains of microorganisms was even more distinct. Only 10–17 per cent “takes” occurred in the former three groups (with S. pyogenes D, E, and SA) compared with 71–98 per cent with the other bacterial strains and 93 and 100 per cent in the controls and chloramphenicol controls. The survival time was not substantially increased in mice in which tumor growth occurred because of viable tumor cells’ remaining after incubation with the bacteria (last column, Part II, Table 1).

Results obtained following intraperitoneal injection of bacteria-treated Sarcoma 37 and Krebs-2 carcinoma were similar to those obtained following subcutaneous injection (compare Table 1 with Table 2), except that the number of “takes” was higher even in the streptococci-treated group. The peritoneal cavity seems to present a more favor-
able environment than the interscapular region for the recovery of the damaged but still viable tumor cells. There is, however, a significant (two- to three-fold) increase in survival time in the groups of mice receiving the streptococci-treated tumor cells intraperitoneally compared with the controls, as would be expected from the microscopical evidence of cell damage. Sarcina lutea and S. faecalis were without effect on the tumor cells, as can be seen from the number of tumor “takes” and survival which were comparable to the controls. No information on the number of “takes” is available for intraperitoneally injected tumor cells incubated with S. marcescens, since we were unable to prevent death within 24 hours of all the injected mice, although chloramphenicol was added to the mixture prior to injection and the ratio of bacteria to tumor cells was lower than for any of the other bacterial strains (65:1). Judging from the phase observations (see Figs. 3 and 9) and from data obtained on the subcutaneously injected group, the ascites tumor cells were not damaged by Serratia marcescens.

Injecting the streptococcal suspensions intraperitoneally with the tumor cells (without prior incubation together) and injection of penicillin 1½ or 3 hours later did not result in a retardation of tumor growth as judged by tumor size and survival time; nor did the intraperitoneal or intratumoral injection of the bacterial suspension in 2- or 7-day-old ascites of 7-day-old solid tumors have any effect on tumors or hosts.1

Persistence of microorganisms in hosts.—The injected bacteria persisted occasionally until the end of the experiment in the treated animals regardless of the bacterial strain used without causing an overt infection (Table 3). Because of the antibiotic treatment there was no apparent effect on the normal host cells, except that several mice which were tumor-free died from streptococcal infection.

Phase Observations

Before the preparations were inoculated into mice, the control tumor cell suspension and the bacteria-treated tumor cells were observed under the phase microscope at the beginning and immediately following the incubation period to observe and record the cytological changes. Some of the typical morphological alterations taking place after different incubation periods with various bacterial strains are shown in Figures 1–11. Figures 1 and 7 show the Krebs-2 carcinoma and Sarcoma 37 control preparations, respectively, which remain essentially unchanged after 1½–2½ hours’ incubation with Mammalian Ringer. In addition, time-lapse photographic studies carried out for 24 hours revealed no morphological changes in control tumor cells (6). Krebs-2 cells incubated with S. faecalis (Fig. 2) and S. marcescens (Fig. 3) are indistinguishable from the control cells. In contrast, cells exposed to S. pyogenes for only 25 minutes become enlarged, show swelling, extensive granulation of the nucleus, and emission of small and large clear blebs from the cytoplasm (Fig. 4). S. pyogenes D and S.A produce similar changes (Figs. 5, 6). A greatly enlarged tumor cell can be seen in Figure 5. Its diameter is approximately 45 µ compared with 10–17 µ for the control cells. Vacuoles which are stainable with Sudan red, indicating the presence of fat globules, become apparent (Figs. 4–6). Staining of streptococci-treated tumor cells after fixation in ether alcohol with hematoxylin confirmed the cellular disintegration.

The same pattern is apparent for Sarcoma 37: the bright halo of the control cells is a conspicuous sign of an intact cell membrane (Fig. 7). It can be seen that S. faecalis and S. marcescens produce no visible effects on tumor cells (Figs. 8, 9). The dots and blurs apparent on S. marcescens pictures (Figs. 8, 9) are due to the constant motion of the bacterial rods.

In a time-lapse photographic study (6) of Streptococci and Sarcoma 37 and Krebs-2 carcinoma cells the first cells lysed minutes after mixing of the bacteria and tumor cells, whereas the last cell lysed from 40 minutes to 4 hours, depending on the ratio of bacteria to tumor cells. The lysis was preceded by nuclear contraction and cytoplasmic swelling with massive emission of blebs, which increase in size and often envelop the entire tumor cell. The swelling was followed by a sudden rupture and contraction of the cell.

Control cells maintained their structural integrity and continued the emission and retraction of thin filopodia and ameboid motion for the period of observation (24 hours). Leukocytes remained motile in the control preparations, whereas in tumor cell preparations mixed with streptococci leukocytes were quickly immobilized or destroyed.

Tissue culture studies.—Since it has been demonstrated that S. pyogenes S.A was cytotoxic to tumor cells, it was considered desirable to test the effect of the bacterial suspension on normal cell populations. Monolayer cell cultures of nonmalignant origin—i.e., of mouse embryos—were subjected to the action of bacterial suspensions in a series of dilutions and for varying periods of time. The number of cells remaining attached to the substrate, compared with controls, was taken as a measure of the cytotoxic effect of the bacterial suspension. Two bacterial strains were tested: S.

1 Unpublished laboratory data.
**Histopathology**

The tissue studies on mice given subcutaneous implants of incubated mixtures of Sarcoma 37 and bacteria indicate that within 24 hours the tumor cells are surrounded by a more intense inflammatory reaction than occurs in ordinary tumor implants (Figs. 12, 14, 16). Despite this some sarcoma cells are considered still morphologically viable among the majority of obviously necrotizing tumor cells at the 24-hour period. In successive periods up to 1 week the inflammatory reaction increases with abscess formation and necrosis. Although the lesions from bacteria-treated implants at 7 days are comprised of tumor cells, cellular debris, and inflammatory exudate, the fact that they are consistently smaller (Tables 1, 2) than the controls indicates that the number of tumor cells is greatly reduced. No recognizably viable tumor cells were found after the first 24 hours in the mice studied, but our data indicate that some cells occasionally survive in a few animals, as evidenced by a certain number of actively growing implants. There are no obvious histological differences in the reaction patterns induced by the tumor implants containing streptococci and those containing *Serratia marcescens* (Figs. 13–17), although the end-results indicate that the latter is more often accompanied by recovery and growth of the tumor. From the end of the 1st week to the 3rd week the tissue studies indicate a phase of chronicity with healing fibrosis and slow resolution of the necrotic and inflammatory lesions. By the 3rd week the untreated control tumor implants have, of course, become sizable, actively expanding tumor masses. The control mice that received comparable doses of bacteria only were free of demonstrable lesions.

**DISCUSSION**

Several strains of hemolytic streptococci exert a cytotoxic effect on tumor cells in vitro, and this property appears to be strain-specific. Only three out of eight streptococcal strains tested produced cell damage consistently, whereas other bacterial strains (*Sarcina lutea, Streptococcus faecalis*, *S. marcescens*) had little effect on ascites tumor cells.

The three streptococcal strains were equally cytotoxic against Sarcoma 37 and Krebs-2 carcinoma cells. Generally, a greater number of "takes" occur following the intraperitoneal injection of the tumor cells, as compared with the subcutaneous route. It is apparent that host resistance can cope with a small number of bacteria-resistant tumor cells more effectively when they are injected into a localized area such as the intrascapular region than in the peritoneal cavity where they can disseminate freely and where the presence of even a small number of viable tumor cells gives rise to ascites tumors. Another difference between the two routes of injection is a two- or threefold increase in survival time of the mice given bacteria-treated cells intraperitoneally, as compared with that in the controls. No such increase in survival time occurred in mice injected with treated cells subcutaneously. An analogous situation exists when transplanted Sarcoma 37 or Krebs-2 carcinoma respond temporarily to toxin therapy. The inherent rapid growth rate of the tumor soon supersedes destruction of even an extensive part of the tumor, and sizes similar to the controls are attained after 1–2 weeks (5, 8).

Some workers found Streptococci to have a beneficial effect on established tumors: Koshimura and his colleagues reported a favorable response of solid Ehrlich carcinoma to infection with *S. pyogenes SA*, and independently Christensen in another laboratory reported that infection by streptococci reduced the number of metastases in rabbits bearing Brown-Pearce carcinoma (9). Jordan et al. (9) prolonged the survival of mice by infecting them with hemolytic streptococci 48 or 72 hours after nasal instillation of leukemic cells. Some of these mice remained tumor-free for 30 days, when experiments were terminated. In our laboratory the infection of tumor-bearers carrying solid or ascites tumors (Sarcoma 37, Krebs-2 carcinoma, or spontaneous tumors) with *S. pyogenes* strains (including Dr. Koshimura's SA strain) produced no inhibition of growth of the implants or prolongation of survival. In part these differences...
may be attributable to the different tumor systems employed, but other factors might be involved which are not clear at this time. However, our results confirm the cytotoxicity in vitro of hemolytic streptococci described independently by Koshimura et al. and Ginsburg and Grossowicz (4, 10).

The lack of cytotoxicity of the living or heat-killed S. marcescens culture in this test system demonstrates that the regression of established tumors produced by the crude heat-killed culture or its purified entotoxin (3, 5, 8) is host-mediated and does not result from direct cytotoxicity of these products for tumor cells.

While tissue culture studies indicated that streptococcal strains are toxic for monolayers of mouse embryo tissue cells, the cytotoxicity was not correlated with the effect on tumor cells, because even strains which were ineffective against the latter were cytotoxic for tissue culture cells. Isolations performed on the heart blood of all animals indicated that at times microorganisms persisted until the end of the experiment (90 days), without causing an overt infection. This persistence of microorganisms is well apparent from Table 3 and answers, albeit in an indirect way, the question of cytotoxicity of these bacteria for the normal host cells.

Ginsburg and Grossowicz (4) reported several hemolysins (CBH, SLO, and SLD) to be cytotoxic for normal as well as tumor cells. We isolated these hemolysins from our streptococcal strains, according to their method, and obtained preparations of low hemolytic activity which were ineffective against tumor cells unless they contained the living streptococci. Since cell damage was produced by streptococcal strains low in hemolytic activity, substances other than hemolysins are capable of inducing these cytopathogenic changes. We have since obtained several highly purified streptolysin preparations from Dr. A. Bernheimer which are high in hemolytic activity and found these also to be highly cytotoxic for Sarcoma 37 and Krebs-2 carcinoma cells. These studies will be presented in detail in a separate paper.

We may conclude that under the conditions of our experiments striking cytopathogenic effects on tumor cells in vitro are produced by hemolytic streptococci. However, treatment of well established spontaneous or transplanted tumors in the intact host with these same streptococcal strains has been ineffective in our hands.

ACKNOWLEDGMENTS

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REFERENCES

Ascites Krebs-2 carcinoma cell suspensions.—The tumor cells were washed twice and suspended in Mammalian Ringer. Time intervals refer to time of incubation at 37° C., after resting cell suspension of the bacteria had been added to tumor cells. Arrows point to some of the streptococcal chains. All pictures are bright-contrast phase of the unstained preparations. Mag. X700.

Fig. 1.—Krebs-2 carcinoma controls, 1 hour, 35 minutes later.

Fig. 2.—Krebs-2 carcinoma cells and S. faecalis, 2 hours, 5 minutes later.

Fig. 3.—Krebs-2 carcinoma cells and S. marcescens, 2 hours, 5 minutes later.

Fig. 4.—Krebs-2 carcinoma cells and S. pyogenes E, 23 minutes later.

Fig. 5.—Krebs-2 carcinoma cells and S. pyogenes D, 30 minutes later.

Fig. 6.—Krebs-2 carcinoma cells and S. pyogenes SA, 1 hour, 35 minutes later.

Ascites Sarcoma 37 cell suspension.—The tumor cells (Figs. 7-11) were washed twice and resuspended in Mammalian Ringer. Time intervals refer to time of incubation at 37° C. after resting cell suspension of bacteria had been added to tumor cells. Arrows point to streptococcal strains. All pictures are bright-contrast phase of the unstained preparations.

Fig. 7.—Sarcoma 37 control cells, 2 hours, 30 minutes. X700.

Fig. 8.—Sarcoma 37 cells and S. faecalis, 1 hour, 35 minutes later. X700.

Fig. 9.—Sarcoma 37 cells and S. marcescens, 1 hour, 35 minutes later. X700.

Fig. 10.—Sarcoma 37 cells and S. pyogenes SA, 1 hour, 35 minutes later. X700.

Fig. 11.—Sarcoma 37 cells and S. pyogenes D, 1 hour, 30 minutes (oil immersion). Arrow points to streptococcal chain amid cellular debris. X1700.
Fig. 12.—Untreated Sarcoma 37, 24 hours after implantation. The tumor cells are generally in excellent condition and rather loosely arranged, with an admixture of leukocytes suggesting a moderate reactive inflammatory response of the host. Hematoxylin & eosin (H. & E.), X195.

Fig. 13.—Untreated Sarcoma 37, 48 hours after implantation. The tumor cells are more compact than at 24 hours and are arranged in infiltrating proliferative sheets with localized concentrations of inflammatory cells. H. & E., X195.

Fig. 14.—Sarcoma 37, 24 hours after implantation of the mixture of tumor cells and a resting cell suspension of Serratia marcescens. The tumor cells are degenerated, as evidenced by pyknosis distortion and poor staining propensities. The reactive leukocytic infiltrate is relatively greater around the nidus of tumor cells in which there is no evidence of proliferative activity. H. & E., X195.

Fig. 15.—Sarcoma 37, 48 hours after implantation of the mixture of tumor cells and Serratia marcescens. The tumor cells are in a more advanced state of degeneration than those studied after the 24-hour period. Few if any of the residual tumor cells are recognized as possibly viable, and the cellular debris is the locus for a high content of inflammatory cells. H. & E., X195.

Fig. 16.—Sarcoma 37, 24 hours after implantation of the mixture of tumor cells and a resting bacterial cell suspension of Streptococcus pyogenes SA. The tumor cells are degenerated, and clumps of organisms are seen as gray granular material. The inflammatory infiltrate is extensive and contains necrotic debris. H. & E., X195.

Fig. 17.—Sarcoma 37, 48 hours after implantation of the mixture of tumor cells and Streptococcus pyogenes SA. The viability of the relatively few residual tumor cells recognizable in the inflammatory and necrotic mass is questionable. H. & E., X195.
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