Mixed Bacterial Toxins in the Treatment of Tumors

II. Gross and Microscopic Changes Produced in Sarcoma 37 and in Mouse Tissues*

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Mixed bacterial toxins have been used in an effort to destroy malignant tumors in patients and animals since 1892, when Coley (13) initiated clinical applications. No systematic study of the histopathologic effects of mixed bacterial toxins on experimental tumors has been found. Several good studies of changes elicited in tumors by products from single bacterial strains have been published (3, 5, 17), but the specific investigation of changes produced by mixed bacterial toxins in tumors and hosts seemed warranted.

MATERIALS AND METHODS

The mixed bacterial toxins employed were obtained from a 14-day culture of Streptococcus pyogenes and a 7-day culture of Serratia marcescens grown separately and mixed in a 1:1 ratio prior to heat sterilization—E<s>W</s>s<s>t</s>r (22).

Three separate groups of Swiss mice were used for the study of the tissue effects of the toxins. In the first group of 65 normal mice of both sexes, the test animals were given a relatively high dose of 1.5 ml. intraperitoneally to produce general effects comparable to those seen in tumor-bearers given only 0.05 ml. In the second group containing 70 normal mice, a lower dose of 0.05 ml. was used in the test animals, because this was the dose used in treating tumor-bearers. Each group of normal mice was divided into seven subgroups of eight to ten animals. The subgroups consisted of male and female treated mice (seven or eight) and one or two animals for the control pool of 28 normal mice. These subgroups of eight to ten mice were sacrificed for study at each of seven set intervals (1 hour, 3 or 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, and 72 or 96 hours) following the toxin treatment.

The third group of 116 tumor-bearing mice of both sexes included ten subgroups, each containing eight treated animals and three or four untreated controls. The tissue studies on the tumors were extended to include post-treatment intervals of 7, 11, and 21 days. In all, 185 normal mice and 116 tumor-bearing mice were used in these experiments.

The transplantable tumor used was Sarcoma 37, carried in the ascites form in Swiss donor mice until implanted into 8-week-old mice. The inoculum of 0.25 ml. of ascites tumor cell suspension (approximately 3.5 million cells) was given subcutaneously in the interscapular areas. Seven days after implantation, when the growing tumor reached at least 1.5 sq. cm. at its base, the mixed toxins were administered.

Tissues taken at necropsy were fixed in Bouin’s solution, processed routinely for paraffin embedding, and stained with hematoxylin and eosin.

RESULTS

Normal mice given 30 times the therapeutic dose, 1.5 ml. of the crude bacterial toxins—E<s>W</s>s<s>t</s>r—were subject to tissue changes which proved to be definite, regular, and generally reversible. Animals sacrificed in groups of ten at specific intervals from 1 to 72 hours after treatment gave macroscopic evidence of only a slight blush or hyperemia on the pleural and peritoneal surfaces. Slight swelling of some of the lymphoid tissues could also be detected grossly. The histologic changes, limited to the lungs and lymphoid tissues, were definitely recognizable only after 4 hours. The slightly swollen pulmonary alveolar walls of the test animals were infiltrated by neutrophilic leukocytes. At the same post-treatment time a slight hyperemia of the splenic red pulp was evident, and a few of the lymph nodes were moderately hyperplastic with slight evidence of the alarm reaction characterized by the presence of some nuclear debris amidst the cells.

Although the leukocytic infiltration in the lungs reached a peak at 12 hours (Fig. 1), the lymphoid tissue changes characterizing the alarm reaction had not developed fully until after 24 hours, by which time most of the lungs were almost normal again. In the spleen the red pulp was engorged, and the perifollicular zones were the site of neutrophilic infiltration (Fig. 2). Reactive hyperplasia enlarged the lymph nodes, and nuclear debris could be found in the sinusooids and follicles (Fig. 4). The thymus, though slower in developing these changes, was also the site of nuclear fragmentation characterizing the alarm reaction. By 48 hours post-treatment, the lymphoid tissue changes gave no evidence of further distortion and were considered to have abated moderately. Examinations on
apparently recovered mice sacrificed at 72 hours after the test injections suggested a return of the lymphoid tissues toward a normal state, but some evidence of the reaction persisted, particularly in the spleen. Several of these animals examined had developed some chronic inflammatory reaction in the lungs.

In the normal control mice given $0.05$ ml. of the mixed bacterial toxins—$E_{1487(5)}$—no constant macroscopic evidence of tissue abnormality was found. Only spot checks for histological changes were made on each interval group in this part of the experiment because of the dearth of gross changes and the extensive studies on normal mice given the much larger dose ($1.5$ ml.). The lower dose produced only slight tissue changes attributable to the toxins. The minimal toxic effect in normal mice contrasts remarkably with the severe reaction produced in tumor-bearing animals given the identical amount of toxins.

The groups of Sarcoma 37-bearing mice treated with $0.05$ ml. of the crude bacterial toxins—$E_{1487(5)}$—were sacrificed for tissue examinations at post-treatment periods varying from $1$ hour to $21$ days. In these animals the changes produced in the lungs and lymphoid tissues were nearly the same as or very slightly greater than those produced in the normal animals given $1.5$ ml., 30 times the dose. The acute leukocytic infiltration of the pulmonary alveolar walls (Fig. 3) and the cellular fragmentation indicating the alarm reaction in the lymphoid tissues were practically indistinguishable from these same evidences of toxicity seen in the normal mice receiving the larger dose ($1.5$ ml.).

The tumors of the treated animals were compared with those of untreated controls sacrificed simultaneously with the test animals. Macroscopically (Figs. 5–8), there was little to observe in the tumors of treated animals until about $6$ hours after treatment. Between $6$ and $24$ hours, the treated tumors, when opened, were found to be hemorrhagic and generally softer than the controls. At $24$ hours the tumor masses were usually blue-black under the skin and bloody when sectioned. Areas of soft necrosis were apparent, but some firm areas were usually found near the periphery. After this time a dark crust developed at the tumor site, and beneath this was the shrunken hard residuum of tumor or tumor debris. During the period from $2$ days to $4$ or $7$ days post-treatment, there was difficulty in recognizing grossly the presence or absence of viable tumor unless the latter was evident by the residual debris, which consisted of nothing more than a dried black crust. During the 2d week a cure or failure became obvious, because any viable tumor had usually evidenced regrowth by $10$ or $11$ days following therapy. By the 3d week the animals exhibited either a dermal scar indicating complete regression or a large tumor which had regrown to a size comparable to that of the tumors carried by the untreated controls.

At $1$ hour after treatment the tumors were not significantly different histologically from the untreated controls, since the only necrosis found could be considered spontaneous. An attempt was made to differentiate histologically between the spontaneous necrosis so commonly found in transplanted tumors and the degeneration and necrosis that might reasonably be attributed to the agent under test for oncolytic potency. In general, the spontaneous necrosis occurred in more or less large swaths and islands well circumscribed and clearly delineated from the adjacent viable tumor composed of masses of clear, compact, but evenly arranged malignant cells (Figs. 9 and 10). This distribution of necrosis was seen equally well in controls and treated animals. Three hours after treatment the tumors developed rather diffuse histologic changes not seen in the controls and characterized by cellular degeneration, loss of sharp, clear cell outlines, looseness of cells, and intercellular debris or cellular detritus (Fig. 11). With each increase in post-treatment time through $6$ hours, $12$ hours, and $24$ hours the degeneration and necrosis attributable to therapeutic tumorlysis increased so that at the last period the effect could be considered severe in some tumors (Figs. 12 and 13).

The deleterious effects including cell shrinkage, nuclear pyknosis, and, finally, cell detritus were not seen in control animals except in very limited areas near zones of extensive spontaneous necrosis. In none of these treated tumors up to $24$ hours post-injection could the morphologic evidence yet warrant a conclusion that the tumor had lost its viability.

At $48$ hours the extensive and severe degeneration and necrosis of the treated tumors left only one of eight in the group with any good viable tissue, whereas the other seven were so damaged that they could be considered as possibly viable only because of harboring some morphologically intact cells. Four days after treatment no recognizably viable tumor could be found in three of the eight tested animals, and in another three the necrosis was almost complete, so that only two mice bore tumors in which small areas might be considered as possibly viable (Figs. 14 and 15).

From 1 week onward through the 11- and 21-day periods, each group of eight tested animals had either four or five mice free of recognizably viable tumor. As the time advanced, the amount of necrosis and slough decreased in the apparently cured animals, so that at $3$ weeks only dermal and
subcutaneous scars remained in some of the tumor-free mice (Fig. 16). In those animals where some tumor cell viability was retained, the residuum rapidly recovered and achieved a state comparable to that of the tumors in the untreated controls of the same group.

**DISCUSSION**

The mixed bacterial toxins when given to normal mice at relatively high dosages produce a nonspecific alarm reaction. The changes in the lymphoid tissues are morphologically similar to those produced by an effective antigen. The leukocytic trapping which occurs in the pulmonary alveolar walls is probably due to the same mechanism shown to be at work by Bierman et al. (9, 10). This mechanism within the lungs was found to be capable of removing billions of transfused leukocytes. These investigators found that "administration of histamine intravenously causes marked respiratory distress and is associated with a prompt decrease in circulating leukocytes." The leukopenia was shown to be caused by removal of cells in the pulmonary circulation. The alarm reaction is characterized by a transient leukopenia, and it has been found that some bacterial products (bacterial polysaccharide of Serratia marcescens) elicit the same effect followed by leukocytosis. In mice bearing transplanted Sarcoma 37, the same general changes are produced with a much smaller dose of mixed toxins; the tumors undergo degeneration, and more than half are completely destroyed. The hemorrhage and necrosis occurring in the treated tumors are characterized histologically by clouding, cellular degeneration, and edema, followed by cell shrinkage, nuclear pyknosis, and disintegration with eventual absorption of the cellular debris. The earlier changes are similar to those described by Apitz as occurring acutely in Ehrlich carcinomas after treatment with E. coli filtrates (5). Our observations on the actual tumor cell changes were comparable to those described by Andervont (3), but we were not impressed with any accumulation of leukocytes within the capillaries of the tumor. Detailed cytologic studies, as carried out by Diller and Shear after treatment of tumors with S. marcescens polysaccharide (17, 18), were not attempted in our study, but insofar as morphologic comparisons could be made, the general changes produced by the mixed toxins were qualitatively compatible with those elicited by the polysaccharide. A complete explanation of the mechanism of their action has never been generally agreed upon, but it would seem reasonable to think that the mechanisms of oncolysis may be similar for different bacterial products including the mixed bacterial toxins.

A variety of theories has been offered to explain in part or completely the basic cause and pathogenesis of the tumor damage produced by bacterial agents. In the outline given below, there are listed some of the published viewpoints on the factors possibly implicated in the hemorrhage and/or necrosis occurring in treated tumors. No attempt has been made to be all-inclusive in the list, but some effort has been expended in trying to include most of the factors which at one time or another have come under consideration as etiologically significant or important in the pathogenesis of oncolysis by bacterial agents.

1898, Spronck (36).—Apparently the first to use bacterial toxins of Streptococcus erysipelas against tumors. He also tried the killed organisms therapeutically. He states that, up to that time, there were 38 observations in the literature concerning the advantageous effects of erysipelas on tumors, most-ly on sarcomas, and quotes P. Bruns as believing the effect to be a general one, probably based on hyperthermia. Spronck ruled out fever as responsible, because, although infections and high fever were common, beneficial effect was rare. No direct effect of organisms on tumor was considered probable. He believed that parasites or certain microbes caused cancer and that these agents could be damaged by products of other organisms such as streptococci and other bacteria, thus attributing an antibiotic action to the bacterial products.

1911, Coley (13, 14).—First to use mixed bacterial toxins against tumors in 1898 and thought effects were better than with single toxins. Considered the action to be systemic and not directly on tumor cells but possibly involving a complicated immunologial phenomenon. "Toxins may produce antibodies which render unfavorable the conditions necessary for the life and further growth of the tumor cell."

1907, Beebe and Tracy (8).—Cancer cells have a relatively greater instability and susceptibility to toxins than do normal cells.

1912, Woodman (38).—Coley's toxins, relatively harmless to normal mice, as compared with the effect on tumor-bearing animals and carcinomas are refractory. Hemorrhage occurs in treated tumors.

1933, Showtman and Michailovsky (35) and 1936, Showtman (34).—Effect of bacterial toxin on tumor is similar to phenomenon of local skin reactivity to bacterial filtrates (Shwartzman Phenomenon). Transplanted tumors and hosts frequently infected secondarily, and secondary bacterial invaders may cause sensitization for an anaphylactoid or hypersensitivity reaction resulting in hemorrhage, necrosis, and regression of tumor.

1935-1945, Duran-Reynals (19, 80).—Reactions of tumors to bacterial toxins are not due to Shwartzman Phenomenon but to special conditions existing in the vessels supplied by the host for rapidly growing tumors. These conditions render the vessels apt to react with the blood-carried bacterial toxins. Slowly growing spontaneous tumors are not vulnerable to the reaction.

1933, Apitz (6).—Effect is due to Shwartzman Phenomenon but with direct action of bacterial toxin on tumor cells, causing elaboration of necrotic cell products including "necrohormones," which are necessary to the creation of fixed "reaction bodies" sensitizing the tumor. Secondly, the circulating "reaction bodies" of the blood complete the conditions resulting in the localized hemorrhagic lesion.

1936, Andervont (3).—Bacterial filtrates produce rapid in-

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1 A. J. Donnelly and H. S. Bowman, unpublished laboratory data on mouse experiments and clinical investigations.
crease of blood in tumor until vessels become dilated and rupture, causing a diffuse hemorrhage throughout the tumor. Some tumor cells are swelled before the hemorrhage, indicating a direct effect of the bacterial products on the tumor cells. 1937, Boyland and Boyland (11).—Ascorbic acid is responsible for the integrity of intercellular substance of capillaries. The production of hemorrhage in transplanted tumors by bacterial filtrates is accompanied by marked reduction in ascorbic acid content of tumor.

1938, Gerber and Bernheim (21).—Action is directly on tumor cells, with bacterial filtrates failing to produce thrombosis or other vascular changes, despite the report of gross hemorrhage.

1939, Anderront and Shakin (4).—Regression of transplanted tumors after bacterial filtrate treatment is present only when a gross hemorrhage is produced in the tumor, and that regression is directly proportional to the amount of hemorrhage. Bacterial filtrate by suddenly lowering ascorbic acid content of tumor weakens its fragile capillaries with resultant hemorrhagic extravasation. Ascorbic acid prevents hemorrhage and resultant tumor regression.

1943, Barrett (6).—Bacterial agents may act on tumors through anaphylaxis. Histamine also causes hemorrhage and necrosis in tumors. Shock with hemorrhage and necrosis in transplanted S-87 tumors occurs in mice treated with horse-serum antigen after previous homologous sensitization.

1945, Shimkin and Zon (33).—Although bacterial products are known to produce thrombocytopenia, the hemorrhagic phenomenon occurring in treated tumors are not due to depletion of platelets, because antiplatelet serum causing a severe depletion does not induce hemorrhage in tumors.

1946, Beck and Fisher (7).—The oncolytic effect of bacterial polysaccharide in mice is not due to hyperthermia, because although the body temperatures of mice are reduced by the treatment, the tumors still undergo hemorrhage and necrosis.

1947, Alpire, Legallais, and Park (9).—The mechanism of action of the bacterial polysaccharide of S. marcescens is one example of a common host reaction to a variety of agents which similarly decrease the host circulatory rate, secondarily affecting the tumor circulation with resulting irreversible anoxic damage. Stasis and occlusion of vessels in transplanted tumors are followed by diffuse petechial hemorrhages and necrosis.

1947, Diller (17).—The bacterial polysaccharide of S. marcescens destroys cells in mitotic division and suppresses mitosis. As with a mitotic poison, the cells most affected are those in division.

1948, Seligman, Shear, Leiter, and Sweet (31).—After administration of tumor-necrotizing polysaccharide tagged with radioactive iodine, the recipient mice had the greatest radioactivity in the liver, lung, and kidney, decreasing in that order. The tumors examined held little radioactive material, even after a large dose of radioactive iodine, prior to cell division and prevent the protoplasmic gelation necessary to complete mitosis.

1951, Lasfargues, Wharton, and DiFina (25).—The action of the bacterial products tested was not directly on the tumor cells, because cells in tissue culture were not deleteriously affected when exposed to such products.

1955, Campbell, Farr, and Rinderknecht (18).—Supports Barrett by showing that anaphylaxis can induce hemorrhage in transplanted mouse tumors, but also suggests that a specific immune mechanism may be involved in the activity of the tumor hemorrhagic factor from E. coli. Assumes that E. coli or homologous antigens from other bacteria may originate in the intestine. The inhibitory effect of cortisone on anaphylaxis may be basically the same action as the inhibition of cortisone on hemorrhage production in mouse transplantable tumors.

1955, Shear (39) and Perrault and Shear (29).—For the production of hemorrhagic necrosis by bacterial polysaccharides in tumors no preparatory injection is necessary, as it is in the Shwartzman Phenomenon. Endogenous polysaccharide may sensitize a tumor, since animal tumors have yielded polysaccharide materials which possess some biologic properties in common with the bacterial polysaccharides, including a tumor-necrotizing activity.

1956, Pradhan, Acheinstein, and Shear (30).—“The tumor-necrotizing potency of the bacterial polysaccharide from S. marcescens was found to be effective in cortisone, dihydroergotamine, pentobarbital sodium, and phenobarbital-sodium did not show any inhibitory effect.” The tumor-necrotizing effect of the bacterial agent was not related to pressor or depressor effect on the systemic circulation.

“While vasoconstriction appears to play a significant role in some of the phenomena involved in the production, or blocking, of hemorrhagic necrosis in tumors, other observations are not explicable on this basis.”

1957, Aloum and Zahl (1).—Serratia marcescens endotoxin in doses adequate for hemorrhage production in mouse Sarcoma 180 results in significant lowering of ribonucleic acid and adeninonitrophosphate levels in the tumors. The significance of these changes in terms of oncolytic action is not suggested by the authors, but such changes could well be a result of tumor damage rather than a precursor or cause of necrosis.

It should be noticed that a few of the mechanisms thought to be involved in the action of bacterial products on tumors are at variance with other ideas concerning the pathogenesis, but there is a considerable overlapping of compatible ideas among the conclusions. Our own observations would indicate that the hemorrhage commonly encountered in treated sarcomas (Fig. 19) is simply secondary to degeneration and disintegration of tumor cells including those forming the walls of vascular channels. In rapidly growing cellular tumors with meager stromal support and a dearth of true endothelial linings in the vascular slits, any diffuse necrotization is inevitably followed by hemorrhage. Since sarcomas are more or less characteristically vascularized by structurally inadequate channels, their relatively greater susceptibility to bacterial toxins is understandable to some degree.

The mechanism of action by which the toxins produce the tumor necrosis is not evident from the histology alone, because the degenerative changes are not recognized as specific; but there can be no
doubt that tumor necrosis does occur. The theory that certain bacterial agents can act as inhibitors of mitosis is supported by the work of Diller (17) and also by Heilbrun's description of the influences on protoplasmic gelation (23). The latter's views have been corroborated recently by the work of Nishimura and Baum (26), who found the same activity in Serratia polysaccharide and in colchicine. These investigators found that certain ascites tumor cells in mice were affected temporarily by S. marcescens polysaccharide. Later, repeated doses were ineffective, and the failure to produce further growth inhibition by such follow-up therapy was attributed to the development of antibodies which appear in the ascitic fluid and prevent the anti-gelation (27).

Effects on intracellular viscosity and mitosis may be considered as more or less local phenomena. In addition, the evidence for a systemic effect on host-tumor relationships is strong. The broad inclusive statement of Coley that the systemic action may possibly involve a complicated immunological phenomenon (14) has been supported in specific areas of reference by the investigations and conclusions of Shwartzman (34, 35), Duran-Reynals (19, 20), Apitz (5), Barrett (6), Campbell (12), and others. The recent discovery that a newly isolated serum protein, properdin (29), may be involved in a nonspecific natural resistance, and that the constituent agents used singly (22). They suggested that the findings of Creech (16) and Wharton (37) may be evidence that a tolerance or increase in resistance to infection and on properdin levels in animals (24). They have suggested that the findings of Creech (16) and Wharton (37) may be evidence that a tolerance or increase in resistance to the lethal action of an endotoxin may be due to stimulation of the properdin system. If there exists any host resistance to tumor development and growth, it may be mediated through a nonspecific natural resistance, a more or less specific immunological reaction (antigen-antibody phenomenon), or both. In any case the bacterial products that have been used for their tumor-necrotizing qualities could stimulate both such defense mechanisms.

Havas et al. have shown that the mixed bacterial toxins have a greater oncolytic potency than the constituent agents used singly (28). They speculate that the combination of exotoxin and endotoxin may potentiate effects because more than one mode of stimulatory action may be involved. The apparent synergy inherent in the mixed toxins and missing in the purified bacterial polysaccharide justifies continued investigation of this form of biotherapy of cancer and warrants a chemical analysis of the mixture to uncover and purify or concentrate the active oncolytic material or materials.

SUMMARY

1. Mixed bacterial toxins of Serratia marcescens and Streptococcus pyogenes produced necrosis in Sarcoma 37 when given intraperitoneally to Swiss mice bearing the tumor subcutaneously.

2. The toxins acting on Sarcoma 37 produced changes which included edema of cells, pyknosis of nuclei, shrinkage of cells, and cellular disintegration, with resulting hemorrhage and eventual sloughing of the tumor followed by healing and scar formation.

3. The mixed bacterial toxins also caused a general reaction which was much more severe in tumor-bearing mice. The morphologic evidence of this general reaction was relatively nonspecific and consisted of pulmonary and splenic trapping of leukocytes, together with a great increase in the numbers of disintegrating cells in the lymphoid tissues.

4. Consideration is given to possible mechanisms involved in the tumor-necrotizing action of bacterial products, and some speculations are made.

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REFERENCES


9. Birnbaum, H. R.; Byron, B. L., Jr.; Kelty, K. H.; and Cordon, F. The Behavior of Leukemic Cells during Continuous Cross-transfusions between Patients with Leuke-

12. CAMPBELL, D. H.; FAHR, R. S.; and RINDERKNECHT, H.

11. BOTLAND, E., and BOTLAND, M. E. LXII Studies in Tis

10. BIERMAN, H. R.; KELLT, K. H.; CORDES, F.; PETRAKIS,

14. . A Report of Recent Cases of Inoperable Sarcoma

13. COLET, W. B. Treatment of Inoperable Malignant Tumors

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22. HAVAS, H. F.; GHOESBECK, M. E.; and DONNELLT, A. J.

20. . Reaction of Spontaneous Mouse Carcinomas to

17. DILLER, I. C. Degenerative Changes Induced in Tumor

19. DÜRAN-RETNALS, F. Reaction of Transplantable and

stain. X180.

FIG. 2.—Edge of follicle in spleen of normal mouse 24 hours
after treatment with 1.5 ml. of bacterial toxins. Perifollicular
concentration of polymorphonuclear leukocytes is evident in
upper half of photomicrograph. H. & E. stain. X600.

FIG. 1.—Lungs of normal mouse 12 hours after treatment
with 1.5 ml. of bacterial toxins. Leukocytic trapping in alveolar
walls concentrates polymorphonuclear leukocytes. H. & E.
stain. X180.

Fig. 3.—Lungs of tumor-bearing mouse 8 hours after treatment
with 0.05 ml. of bacterial toxins. Leukocytic trapping in
alveolar walls comparable to that seen in Figure 1. H. & E.
stain. X180.

Fig. 4.—Lymph node of normal mouse 24 hours after treatment
with 1.5 ml. of bacterial toxins. Nuclear debris characterizes the
alarm reaction. Similar changes can be seen in the
thymuses of the same animal. H. & E. stain. X600.
Upper photographs are of control mice (A) implanted subcutaneously with ascites Sarcoma 37 at same time as the toxin-treated test animals (B) seen in lower photographs.

Fig. 5.—Mice at time of treatment, with tumors approximately 3 sq. cm. at base and well established 7 days after implantation.

Fig. 6.—Mice in lower photograph 3 days after treatment with 0.05 ml. of mixed bacterial toxins. Controls have tumors about 3.5-3.0 sq. cm. at base, whereas the treated tumors consist of debris and crust.

Fig. 7.—Mice 13 days after start showing untreated control tumors approximately 6-7 sq. cm. at base, and the treated animals in lower photographs. In the latter the tumors have regressed, leaving residual crusts.

Fig. 8.—The untreated control mice have tumors measuring from 11 to 15 sq. cm. at their bases, but at the same period 27 days after treatment the treated tumors have regressed completely, leaving scars.
FIG. 9.—Untreated Sarcoma 37, 7 days after implantation. At this time the tumor was growing vigorously, and histologically the cells are well spaced, sharply outlined, and clearly stained. H. & E. stain. ×270.

Fig. 10.—Same tumor as in Figure 9 but showing swath of spontaneous necrosis sharply demarcated from the good tumor. H. & E. stain. ×270.

Fig. 11.—Sarcoma 37 from mouse treated with mixed bacterial toxins 3 hours earlier. Note loss of clarity of cell outline as compared with that in Figure 9. There is some degeneration, nuclear pyknosis, and cellular debris diffusely scattered, in contrast to the spontaneous changes seen in Figure 10. H. & E. stain. ×270.

Fig. 12.—Sarcoma 37, 6 hours after treatment. Hemorrhage is evident, in addition to degenerative changes which are similar to but more advanced than those seen in Figure 11. It is thought probable that hemorrhage follows the degenerative changes, because the vascular channels or slits lined by tumor cells are disrupted. H. & E. stain. ×270.
FIG. 13.—Sarcoma 37, 24 hours after treatment. More advanced degenerative changes characterized by cellular disintegration to diffuse necrosis. H. & E. stain. X270.

FIG. 14.—Sarcoma 37, 4 days after treatment. No recognizably viable tumor cells are found in this material, which is mostly necrotic and inflammatory. H. & E. stain. X270.

FIG. 15.—The same tumor as in Figure 14 but at lower magnification (X73) to show separating slough of necrotic residuum. H. & E. stain. X73.

FIG. 16.—Residual fibrotic scar of repair following slough of necrotic tumor. H. & E. stain. X270.
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