S^{35} in the cytoplasm of the Kupffer cells of the liver. Similarly, activity of injected biosynthetically labeled S^{35}-RbSA was concentrated in the cytoplasm of lymphoid cells of the spleen, particularly in the red pulp. After injection of lightly labeled S^{35}-sulfanilazo-bovine γ-globulin into the foot pads of rabbits, the radioactive material was concentrated in the cytoplasm of the eosinophilic macrophages of the popliteal lymph nodes. In all these cell types, the protein-bound S^{35} was either present within the cytoplasm or adsorbed to the cellular membrane. Little intranuclear radioactivity was evident.

1. Haurowitz, F., Crampton, C. F., *J. Immunol.*, 1952, v68, 73.

2. Crampton, C. F., Reller, H. H., Haurowitz, F., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 448.

3. ———, *J. Immunol.*, 1953, v71, 319.

4. Crampton, C. F., Haurowitz, F., *ibid.*, 1952, v69, 457.

5. Friedberg, W., Walter, H., Haurowitz, F., *ibid.*, 1955, v75, 315.

6. Warren, S., Dixon, F. J., *Am. J. Med. Sci.*, 1948, v216, 136.

7. Ingraham, J. S., *J. Infect. Dis.*, 1951, v89, 109, 117.

8. Libby, L., Madison, C. R., *J. Immunol.*, 1947, v55, 15.

9. Sabin, F. R., *J. Exp. Med.*, 1939, v70, 67.

10. Robertson, J. S., *Austral. J. Biol. a. Med. Sci.*, 1952, v30, 59.

11. Latta, H., Gitlin, D., Janeway, Ch. A., *A.M.A. Arch. Path.*, 1951, v51, 260.

12. Gavosto, F., Ficq, A., *Nature*, 1953, v172, 406.

13. Walter, H., Haurowitz, F., Fleischer, S., Lietze, A., Cheng, H. F., Turner, J., Friedberg, W., *J. Biol. Chem.*, 1957, v224, 107.

14. Heidelberger, M., Kendall, F. E., *J. Exp. Med.*, 1935, v62, 697.

15. Hawkins, J. D., Haurowitz, F., *Biochem. J.*, 1959, v72, 5p.

16. McMaster, P. D., Kruse, H., *J. Exp. Med.*, 1951, v94, 323; 1954, v100, 341.

17. Garvey, J., Campbell, D. H., *J. Immunol.*, 1956, v76, 36.

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Necrotizing Effects of *Staphylococcus aureus* Extract on Mouse Sarcoma 180.* (26249)

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In susceptible hosts endotoxins of gram-negative bacteria cause a variety of biological effects, such as fever, local and systemic Shwartzman reactions, necrosis of experimental tumors, as well as alterations of nonspecific resistance, antibody formation, and dermal reactivity to epinephrine(1-5). The lipopolysaccharide moiety of the bacterial endotoxins seems to be responsible for the majority or all of these biological effects. In contrast to the extensive data reported on endotoxins from gram-negative bacteria, only limited information is available on similar biological effects by products from gram-positive microorganisms(3,6,7).

Recently it was shown that a trichloracetic acid extract from *Staphylococcus aureus* strain D alters dermal reactivity of rabbits to epinephrine(8). This effect was comparable to that observed with endotoxins of gram-negative bacteria(5) and the lipid A component of *E. coli* endotoxin(9). In view of this observation, a study of the possible tumor-necrotizing effect of the same staphylococcal extract was undertaken. As reported herein, this extract causes extensive hemorrhagic necrosis of mouse sarcoma 180.

Materials and methods. Two strains (D and 5550) of coagulase positive *Staphylococcus aureus* were used for preparation of extracts, carried out as previously described (8). The extracts were prepared at Difco Laboratories, Detroit, and obtained through

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Unless otherwise specified, mouse sarcoma 180 tumors (S-180) were implanted in HaICR Swiss mice weighing between 17 and 24 g and measured according to procedures already reported(10). Single intraperitoneal injections of the bacterial extracts were given on 7th day after tumor implantation. The necrotizing effects were observed 24 hours later, and usually were evaluated on tumors exposed as shown in Fig. 1; the effect was considered either positive or negative. Results of histological examinations, performed only in a few experiments, were consistent with the visual evaluation.

Results. Intraperitoneal injection of 10 μg of extract from *Staphylococcus aureus* strain D into HaICR Swiss mice bearing a 7 day-old S-180 caused marked hemorrhagic necrosis of the tumor and of the overlying skin (Fig. 1).

TABLE I. Necrotizing Effects of Staphylococcal Extract D on S-180.

7th day after tumor implantation			8th day after tumor implantation
Avg body wt, g	Avg tumor diam., mm	Extract, μg/mouse	No. with hemorrhagic necrosis/Total
18.8	10.0	0*	3/20
19.3	9.8	5	9/20
18.3	11.0	10	11/20
17.3	9.4	20	13/20
18.9	11.0	40	16/20

* Control animals were given equivalent amounts of saline solution.

Histological examination of the treated tumors revealed extensive, almost total necrosis, with marked edema. When the tumor was growing very close to the skin, necrosis and picnosis of nuclei in the epidermis were present (Fig. 2). In addition, picnosis was seen in the Malpighian follicles of the spleen and small foci of necrosis in the regional lymph nodes corresponding to the tumor.

The effects of various amounts of the staphylococcal extract, given to HaICR Swiss mice bearing a 7 day-old S-180, were determined. Incidence of necrosis is related to the amount of extract injected (Table I). Injection of as little as 5 μg per mouse was sufficient to cause an effect. In parallel experiments it was found that the extract was as effective as purified lipopolysaccharide from *E. coli*.

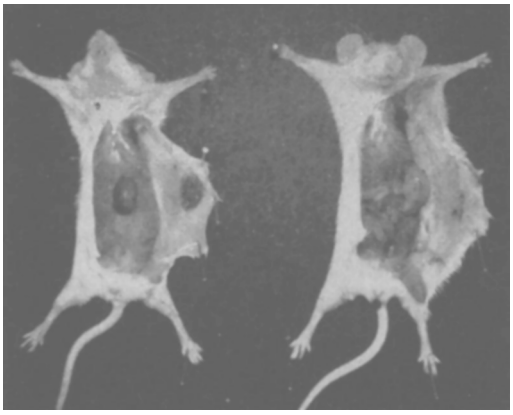

FIG. 1. Effects of extract from *Staphylococcus aureus* D on solid S-180. Left: mouse treated with 10 μg of extract. Right: mouse treated with saline.

In additional experiments, the bacterial extract was injected at various times after tumor implantation (Table II). This treatment had no effect on the tumor when given on the 1st day after implantation and only a slight effect when given on the 4th day.

Experiments with rabbits have revealed that the adrenolytic drug dibenzyline prevents development of the reaction to epinephrine elicited by the staphylococcal extract (8). For this reason, the possible influence of dibenzyline and of epinephrine on the effects of the bacterial extract on S-180 was tested in 2 experiments. Also, the observation of the antagonistic effect of cortisone on several phenomena induced by endotoxins from gram-negative bacteria(1) prompted

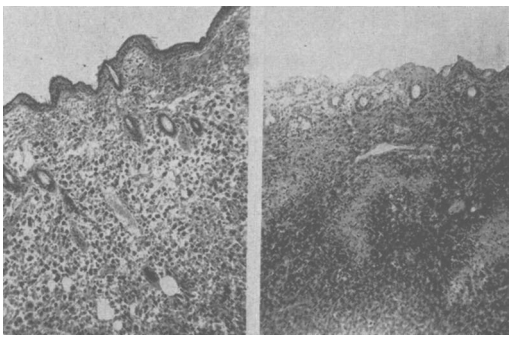

FIG. 2. Histological section of S-180 and overlying skin of mouse treated with 10 μg of staphylococcal extract (left) or with saline (right), 100 ×.

TABLE II. Necrotizing Effects of Staphylococcal Extract D Injected at Various Times after S-180 Implantation.

7th day after tumor implantation		Treatment		No. with hemorrhagic necrosis/Total
Avg body wt, g	Avg tumor diam., mm	Days after implantation	Extract, μg/mouse	
19.8	11.3	7	0*	0/26
19.8	11.9	1	20	0/20
20.3	11.8	1	40	0/30
20.6	12.0	4	20	0/20
19.7	12.2	4	40	7/30
20.0	11.2	7	20	16/20
19.5	11.8	7	40	22/30

* Control animals were given equivalent amounts of saline solution.

the study of the possible influence of this hormone on the tumor necrotizing effect of the *S. aureus* extract. The results of these experiments are shown in Table III. Epinephrine or dibenzyline, at the doses tested, did not modify the tumor necrosis induced by the staphylococcal extract. A reduction in incidence of necrosis was observed, however, when cortisone was given daily for the 7 days between tumor implantation and injection of the extract. This antagonistic effect was not evident when the hormone was administered prior to tumor implantation or starting only 3 days before administration of the bacterial extract. Benadryl was tested in 2 experiments, in view of the possibility that this anti-histamine drug might antagonize histamine-mediated effects of endotoxins(11). Treatment of mice with 10 to 30 mg/kg of Benadryl, 30 minutes prior to administration of the bacterial extract, did not significantly reduce incidence of tumor necrosis. In 2 additional experiments total body irradiation of the host mice (350 r/mouse, in air), given on the day before tumor implantation, did not reduce incidence of necrosis induced by the extract.

S-180, although carried routinely in Swiss albino mice, can grow also in C57Bl/6, DBA2, AKR and C3H inbred mice, even though it regresses completely after initial growth in a large percentage of animals of the latter 2 strains. It was of interest to determine whether the necrotizing effect of the *S. aureus* extract on S-180 could be seen in these inbred strains of mice, regardless of their capacity to reject the tumor. The extract was effective in all strains (Table IV).

In 2 experiments, 20 or 40 μg of the extract had no effect on S-180 ascites in Swiss mice. Neither were hemorrhages noted in the peritoneal cavity when the tumor had grown in ascites form, nor was hemorrhagic necrosis seen in the tumor grown in solid form following subcutaneous inoculation of 10 x 10^6 cells per mouse.

The extract from *Staphylococcus* strain 5550, which failed to elicit the epinephrine

TABLE III. Alterations of Necrotizing Effects of Staphylococcal Extract D by Various Drugs.

7th day after tumor implantation		Treatments			8th day after tumor implantation
Avg body wt, g	Avg tumor diam., mm	Extract, μg/mouse	Compound* and dose, mg/kg		No. with hemorrhagic necrosis/Total
23.0	8.7	0	None	0	0/20
22.4	8.8	20	"	0	13/20
23.4	8.7	0	Dibenzyline	0.5	0/20
22.4	8.8	0	Epinephrine	0.25	0/20
22.1	8.8	20	Dibenzyline	0.5	14/20
21.0	8.5	20	Epinephrine	0.05	11/19
23.3	14.8	0	None	0	0/10
22.2	12.9	20	"	0	10/10
21.0	15.7	0	Cortisone (I, II, or III)	25	0/10
21.6	12.9	20	Cortisone I	25	9/10
20.6	12.7	20	" II	25	3/10
22.4	13.4	20	" III	25	7/10

* Dibenzyline and epinephrine were given intraper. on 7th day after tumor implantation. Cortisone was given subcut. daily for 7 days starting respectively 7 days prior to implantation (I), on day following implantation (II) or on 5th day following implantation (III).

TABLE IV. Necrotizing Effects of Staphylococcal Extract D on S-180 Grown in Various Strains of Mice.

Strain of mice	7th day after tumor implantation: Avg body wt, g	Avg tumor diam., mm	Extract, μg/mouse	8th day after tumor implantation: No. with hemorrhagic necrosis /Total
Swiss	24.0	13.4	0*	0/20
"	21.0	12.8	20	12/20
"	22.5	12.2	40	17/20
C57Bl/6	25.8	11.4	0*	0/9
"	24.7	11.1	20	4/10
"	24.9	11.9	40	8/10
DBA_2	15.4	11.6	0*	0/5
"	16.2	11.2	20	6/9
"	19.8	11.8	40	8/10
AKR	20.8	10.5	0*	1/10
"	20.9	7.5	20	5/10
"	19.7	8.8	40	9/10
C_3H	18.7	12.0	0*	0/10
"	21.5	11.7	20	7/10
"	20.6	14.0	40	6/10

* Control animals were given equivalent amounts of saline solution.

reaction in rabbits(8), also had no tumor necrotizing effect on S-180 solid in Swiss mice in amounts up to 40 μg per mouse.

Discussion. It has long been known that endotoxins of gram-negative bacteria produce hemorrhagic necrosis in certain experimental tumors. This reaction is elicited without preparatory injection of endotoxin into the tumor; in contrast, 2 injections of endotoxin are needed for production of the local Shwartzman reaction. The present study has revealed that intraperitoneal administration of a trichloracetic acid extract from a coagulase positive strain of *S. aureus* into Swiss mice bearing 7 day-old S-180 causes marked hemorrhagic necrosis of the tumor and of the overlying skin. That gram-positive bacteria may contain a component with activities similar to those of endotoxins of gram-negative microorganisms was not entirely unexpected, since endotoxin was obtained also from hemolytic streptococci by Stetson(6). Nonetheless, the possibility has to be considered as to whether the staphylococcal extract is contaminated with endotoxin from gram-negative bacteria. The following observations strongly suggest that this is not the case: (a) the staphylococcal extract had activity of the order of that of endotoxin from gram-negative bacteria, both in eliciting hemorrhagic necrosis in S-180 and in altering the reactivity to epinephrine of rabbits(8); (b) Jensen's staphylococcal antigen A(12), prepared from a different strain in another laboratory, was found to alter reactivity of the rabbit to epinephrine (Jensen and Neter, unpublished data); (c) altered reactivity to epinephrine was produced in rabbits also by injection of viable staphylococci under experimental conditions which preclude contamination with gram-negative bacteria or their endotoxins(8).

In the present investigation the staphylococcal extract did not cause hemorrhagic necrosis in mice bearing 1 or 4 day-old tumors, in contrast to its effect in animals bearing 7 day-old neoplasms. The fact that vascularization of S-180 is adequate only by the 4th or 5th day after implantation(13) suggests that vascularization is necessary for the extract to be effective. This suggestion is supported also by the observation that endotoxin from gram-negative bacteria is not active on tumor tissues *in vitro*(14). The fact that the *S. aureus* extract was not active on S-180 ascites is not sufficient *per se* to support the above mentioned view, because the extract was not effective even when the ascites tumor was grown subcutaneously in solid form. Strain-specific, host defense mechanisms do not appear to be involved in the necrosis of S-180 caused by the extract, since necrosis occurred also in inbred strains of mice, regardless of their capacity to reject S-180 implants.

Small scattered areas of necrosis are noted microscopically in untreated 7 day-old S-180, which increase progressively and become massive 2 to 4 weeks after implantation. The question arises, therefore, whether the marked effects of the bacterial extract in 1 week-old S-180 is related to the necrotic processes which occur in older tumors. The possibility may be considered that a physiological mediator may be operative in either spontaneous or extract-induced necrosis of S-180. Data reported here indicate that epinephrine is probably not an essential mediator in the necrosis caused by the extract.

In fact, this effect was neither enhanced by epinephrine nor antagonized by dibenzyline, a potent adrenergic blocking agent, when these drugs were used in doses effective in rabbits(8). Similarly, the observation that Benadryl, an antihistaminic agent, did not significantly antagonize the effects of the extract on S-180 suggests that histamine does not play a critical role in the pathogenesis of the tumor necrosis studied. The antagonistic effect of cortisone, although difficult to explain at present, is consistent with results obtained by others in different systems and suggests possible interference with the effects of endotoxins by this hormone(1).

The observations described herein on the effects of an extract from *Staphylococcus aureus* on S-180 provides additional evidence that gram-positive bacteria may contain a substance with biological activities similar to those of endotoxins of gram-negative microorganisms.

Summary. 1) A trichloracetic acid extract of *Staphylococcus aureus,* strain D (5 to 40 μg/mouse), in 24 hours caused extensive hemorrhagic necrosis of Sarcoma 180 in HaICR Swiss mice when injected intraperitoneally 7 days after tumor implantation. The extract was ineffective against tumors 1 to 4 days after implantation. 2) The effect was also produced in Sarcoma 180 grown in C57Bl/6, C3H, AKR and DBA_2 mice. 3) Epinephrine, dibenzyline and the antihistaminic drug Benadryl, in the doses used, did not alter the necrotizing activity of the extract. Cortisone, however, reduced the incidence of the effect.

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1. Thomas, L., *Ann. Rev., Physiol.,* 1954, v16, 467.
2. Springer, G. F., editor, *Polysaccharides in Biology.* Josiah Macy, Jr. Foundation, New York, 1957.
3. Bennett, I. L., Jr., Cluff, L. E., *Pharmacol. Rev.,* 1957, v9, 427.
4. Atkins, E., *Physiol. Rev.,* 1960, v40, 580.
5. Thomas, L., *J. Exp. Med.,* 1956, v104, 865.
6. Stetson, C. A., Jr., *ibid.,* 1956, v104, 921.
7. Braude, A. I., McConnell, J., Douglas, H., *J. Clin. Invest.,* 1960, v39, 1266.
8. Neter, E., Anzai, H., Gorzynski, E. A., *J. Inf. Dis.,* 1960, v107, 332.
9. Neter, E., Anzai, H., Gorzynski, E. A., Westphal, O., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 783.
10. Mihich, E., Nichol, C. A., *Cancer Research,* 1959, v19, 279.
11. Greisman, S. E., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 628.
12. Jensen, K., *Acta allergologica,* 1959, v13, 89.
13. Clarke, D. A., Reilly, H. C., Stock, C. C., *Antibiot. and Chemother.,* 1957, v7, 653.
14. Shapiro, C. J., *Am. J. Hyg.,* 1940, v31, 114.

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Role of the Sex Hormones in Peptic Ulcer.* (26250)

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Recent clinical observations have stimulated interest in the relationship of abnormalities of the various endocrine organs and occurrence of acid-peptic ulceration. It has been well established clinically that duodenal ulcer occurs much more frequently in males than in females. A recent report of peptic ulcer occuring in 2 brothers with primary testicular atrophy(1) is of unusual interest. Peptic ulceration is observed frequently in patients with hepatic cirrhosis(2). During pregnancy progressive decrease in gastric secretory volume is followed by a lower incidence of peptic ulcer(3). Pregnancy and cirrhosis are associated with an increase of circulating estrogens.

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