

PHARMACOLOGIC AND ANAPHYLACTOGENIC PROPERTIES OF
STAPHYLOCOCCUS PROTEIN AND UNFRACTIONATED EXTRACT
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A STUDY of the cutaneous reactions to staphylococcus extract and various protein and polysaccharide fractions has been previously reported from this laboratory.¹ We are now reporting preliminary studies on the pharmacologic and anaphylactogenic properties of a whole unfractionated extract and two protein fractions of staphylococcus.

Previous workers have utilized, for the most part, culture filtrates which vary considerably with methods of culture, and which may contain interfering substances.² In the present work, which is part of a study of the role of staphylococcus in bacterial hypersensitivity, we have employed certain chemical fractionated derivatives and whole extracts from ground washed bacteria.

MATERIALS AND METHODS

Two strains of staphylococcus, one pathogenic (F-10) and one nonpathogenic (F-7) were the sources of the material used in this study.¹ In order to eliminate potential antigens from the media as far as possible, the whole unfractionated extract, consisting of an aqueous suspension of the lyophilized and ground, washed bacterial mass was prepared from a 48 to 72 hour culture grown in a synthetic media, modified from Bernheimer and associates,³ and passed through a Seitz filter. From the whole extract one of the two protein fractions was precipitated at pH 3.5. This actually consists of one fraction precipitable at pH 5.3 and one precipitated at pH 3.5, referred to as Fraction A-1 and A-2 in a previous report.¹ Since these fractions demonstrated cross reactivity on precipitin tests,¹ they were combined in the present work and referred to, as fraction A. The second protein fraction, referred to as fraction B, was precipitated with trichloroacetic acid after removal of fraction A. All fractions were dialyzed against a bicarbonate buffer, pH 8.0, before use.

The whole extracts and protein fractions of both F-10 and F-7 strains of staphylococcus were investigated with regard to their pharmacologic effects upon normal guinea pigs and isolated suspended segments of guinea pig ileum. Investigations of anaphylactogenic properties were undertaken on animals which had received one to three months previously, a series of injections of the whole extracts and fractions in an attempt to bring about sensitization. Extracts and fractions were also studied from the standpoint of heat stability.

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Sensitization to the whole extract and protein fractions of strains F-10 and F-7 was attempted in 21 male guinea pigs each weighing between 250 and 300 grams. Four animals received whole extract (F-10), 5 animals received fraction A (F-10) while 4 animals received fraction B (F-10) in four consecutive daily intradermal injections totaling 1.0 mg. of phosphotungstic acid precipitable nitrogen, hereafter termed "protein nitrogen."

Three animals received 1.0 mg. protein nitrogen of whole extract (F-7) intravenously, followed by three daily intradermal injections of 0.1 mg., totaling 1.3 mg.

Three animals received 0.7 to 2.5 mg. protein nitrogen of fraction A (F-7) intravenously, followed by three consecutive daily injections of 0.13 mg. intradermally, totaling 1.0 to 3.0 mg.

Two animals received fraction B (F-7) in four consecutive daily intradermal injections totaling 0.24 mg. of protein nitrogen.

The initial intravenous injections in groups receiving whole extract (F-7) and fraction A (F-7), respectively, were given to study the effect, if any, of these materials in normal animals. Since there was no effect, subsequent intradermal injections were given in an attempt to bring about sensitization.

To test for anaphylaxis, four to twelve weeks after the last sensitizing injection of extracts or fractions, challenging injections of 0.1 to 0.7 mg. protein nitrogen of the corresponding materials were administered intravenously or intracardiac.

To test for primary toxicity, 19 normal, unsensitized animals received intravenous, intracardiac, or intraperitoneal injections of the extracts or fractions in amounts from 2 to 6 times that employed in the challenging injections in the "sensitized" animals. This group of controls included the groups sensitized to whole extract (F-7) and fraction A (F-7) in which the first sensitizing doses were large intravenous injections.

Autopsies were done on both normal and sensitized animals immediately after death. When recovery seemed probable, usually in 15 to 60 minutes, the animals were killed by a blow on the head.

Tests on isolated guinea pig ileum were made by suspending 1 to 2 inch segments in a 3 ml. bath containing Tyrode solution maintained at 37° C. A given volume of extract or fraction (usually 0.2 or 0.4 ml.) containing 0.02 to 0.06 mg. protein nitrogen was added to the bath and reactions of the segment were recorded on a moving smoked drum. Repeated additions to the bath of the same or other materials were similarly studied with each suspended segment of ileum, the segment being washed three times between each addition. Forty-six segments from 12 normal animals and 83 segments from 14 sensitized animals were tested. The materials were added to the bath in various sequences with different segments from each animal. The criteria for a specific anaphylactic reaction to a given material were: (1) initial contact followed by contraction of the segment within one minute, (2) subsequent contacts associated with loss of reactivity (desensitization), (3) contraction of the segment to histamine after desensitization, and (4) absence of contraction to the material by segments from normal (nonsensitized) animals.

The whole extract of the pathogenic (F-10) strain, when placed in contact with the normal segment of ileum, caused a maximal contraction within about 30 seconds, which persisted for about a minute, after which time the segment relaxed and the tracing returned to the base line (Fig. 3). After washing, subsequent additions of this extract to the bath resulted in progressively diminishing and finally absent contraction. After "desensitization" the segment still reacted normally to histamine. Fraction A (F-10) possessed the identical activity of the whole extract (Fig. 2). This reaction occurred with consistency in both control and sensitized animals (Fig. 1). In addition, "desensitization" to whole extract resulted in loss of reactivity to fraction A (F-10), and conversely "desensitization" to fraction A (F-10) caused loss of reactivity to the whole extract. The reactions produced by the whole extract (F-10) and fraction A (F-10) are similar to those produced by snake venoms^{4, 5} and other toxins.^{6, 7} This toxic effect was not present in fraction B (F-10), nor was it found in extract or fractions of the nonpathogenic (F-7) strain (Fig. 1). This toxic factor as demonstrated on the isolated suspended segment of ileum from normal animals was heat stable, withstanding boiling for 15 minutes.

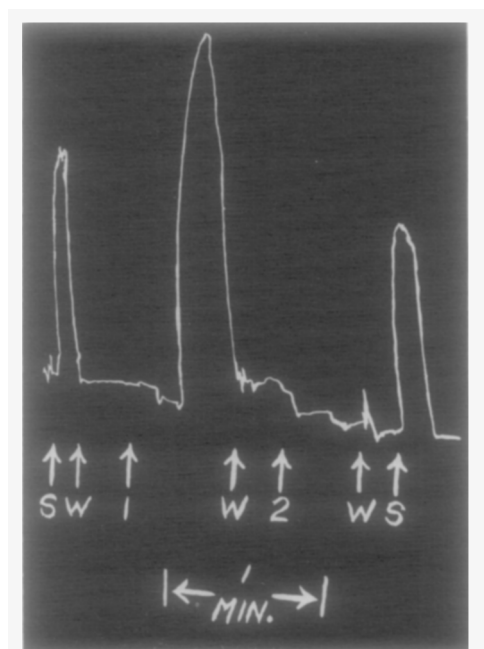


Fig. 2.—Effect of fraction A of pathogenic strain (F-10) upon isolated suspended segment of ileum from a normal guinea pig. S indicates addition of 0.04 μ g. of standard histamine base; W indicates 3 washes.

Arrow 1, 0.025 mg. P.N. fraction A (F-10) added to bath. Maximal contraction after 30 seconds.

Arrow 2, 0.05 mg. P.N. fraction A (F-10) added to bath. No contraction in one minute (desensitization).

Therefore, though the number of animals is small, it would seem that the whole extract and fraction A (F-10) of the pathogenic staphylococcus has an anaphylactoid effect both in the intact animal and the isolated ileum of normal guinea pigs.

TABLE I. ANALYSIS OF DIALYZED PROTEIN FRACTIONS OF PATHOGENIC (F-10) AND NONPATHOGENIC (F-7) STRAINS OF STAPHYLOCOCCUS

	A (F-10)	B (F-10)	A (F-7)	B (F-7)
Total nitrogen ^a	12.54	12.35	13.71	10.00
"Protein" nitrogen ^b	10.64	12.13	13.30	10.00
Total phosphorus ^c	3.68	1.26	2.10	0.00
Inorganic phosphorus ^c	0.12	0.00	0.00	0.00
Carbohydrate (as pentose or glucose) ^d	14.70	7.80	10.80	1.47
Purine bases (qualitative) ^e	+	0	+	0

^aMicro Kjeldahl n; Kabat and Meyer: Experimental Immunochimistry.

^bPhosphotungstic Acid precipitated N—Modified from Cooke, Allergy in Theory and Practice.

^cFiske-Subbarow: J. Biol. Chem. 66: 375, 1925.

^dDische, Alpha-Naphthol Method—Biochem Z. 175: 371, 1926.

^eHawk and Bergheim—Practical Physiological Chemistry, Qualitative test for purine bases.

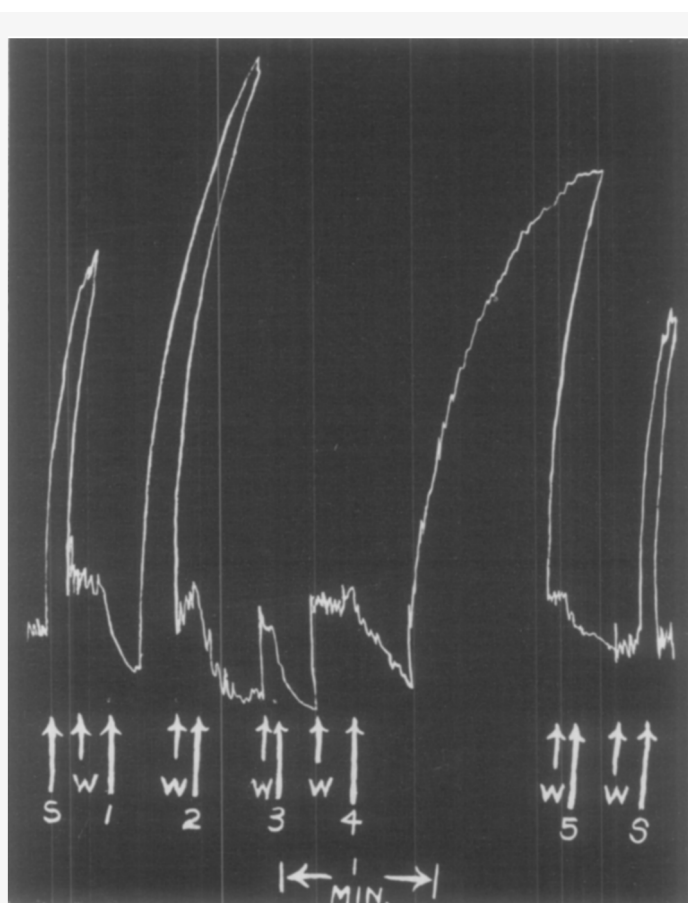


Fig. 3.—Effect of unfractionated extract and fraction B of the pathogenic strain (F-10) of staphylococcus upon isolated ileum from guinea pig sensitized to fraction B (F-10). S indicates addition of 0.04 μ g of standard histamine base, W indicates 3 washes.

Arrow 1, 0.025 mg. P.N. extract (F-10); maximal contraction after 30 seconds.

Arrows 2 and 3, 0.05 mg. P.N. extract (F-10). No contraction in one minute (desensitization to extract F-10).

Arrow 4, 0.028 mg. P.N. fraction B (F-10). Maximal contraction after 30 seconds.

Arrow 5, 0.056 mg. P.N. of fraction B (F-10). No contraction in one minute (desensitization).

Anaphylactogenic Effects of Extracts and Fractions.—Anaphylaxis was demonstrated either in the intact animal or isolated ileum or both with whole extracts and all fractions from both pathogenic and nonpathogenic strains (Figs. 1 and 3). However, since whole extract (F-10) and fraction A (F-10) produced anaphylactoid reactions in the control animals, true anaphylaxis cannot be claimed for these two groups. The three animals sensitized and challenged with fraction A (F-10) showed no reactions, whereas this fraction produced anaphylactoid symptoms in three control animals. A few cross reactions occurred, concerning which no conclusions are warranted until further studies are done.

Anaphylaxis demonstrated in the isolated, suspended ileum of guinea pigs sensitized with the nontoxic extract or fractions was unaffected by previous "desensitization" to the toxic extract or fraction A (F-10) (Fig. 3). However, the anaphylactoid reaction to whole extract (F-10) or fraction A (F-10) was frequently lost following desensitization with specific antigen in segments from sensitized animals.

SUMMARY AND CONCLUSIONS

The pharmacologic and anaphylactogenic effects of extracts and two protein fractions of staphylococcus in guinea pigs and isolated segments of guinea pig ileum have been studied.

The unfractionated extract of a pathogenic strain of staphylococcus produced anaphylactoid reactions in both the intact animal and the isolated ileum. This toxic activity was limited to one of the two protein fractions and was heat stable.

Neither extract nor fractions of the nonpathogenic strain of staphylococcus demonstrated toxic activity.

Since extracts and fractions were obtained from organisms washed free of filtrate, it is concluded that the toxic factor is somatic in origin. Results of chemical analysis suggest that the toxic protein fraction contains nucleoprotein.

Anaphylaxis was demonstrated with all extracts and fractions of both strains of staphylococcus with the exception of the unfractionated extract and the toxic protein fraction of the pathogenic strain. True anaphylaxis could not be proved with these latter two materials because of their primary toxicity. This toxic factor, which may mask anaphylaxis, must be taken into consideration in any study of hypersensitivity to this organism.

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