The manifestations of this disease are almost surely peripheral in origin for certain muscles are electrically inexcitable during an attack. Attention is thus directed to an abnormality of the muscle itself as a cause of the weakness. The facts so far known could be explained on the assumption that from time to time some alteration in the muscle arises which leads to inexcitability and which then requires additional K for its correction. This need is partially met by diffusion of K from serum into muscle as might be concluded from the fact that serum K falls in a severe attack. The increased requirement is not adequately met, however, unless K can be absorbed from the gut. McEachern's observation[7] that the intravenous administration of KCl in small amounts can partially relieve the seizure without raising the lowered serum K could be explained on the basis of direct diffusion of the administered K into the muscle with resulting relief of the weakness.

In this patient the need for extra K is more or less constant for he develops weakness whenever there is no source of available K in the gut. Indeed the urgency of this need is shown by the observation that the washing out of large amounts of K during a simple water diuresis results in a severe attack. Apparently he is unable to compensate for the reduced concentration of muscle salts which follows ingestion of large quantities of water. In other individuals who have infrequent attacks the metabolic change in muscle postulated above must occur only periodically.

The K taken into the muscle during the attack should, on this hypothesis, be eliminated after the abnormality has been corrected. Experiments to check this point are in progress.

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Immunological Properties of a Sonic Extract of Pneumococci.

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Formalin-killed, young cultures of pneumococci have been found by one of us (Weil)[1] to evoke infiltration when injected intra-

[7] McEachern, D., personal communication.

[1] Weil, A. J., and Phillips, S. W., *J. Immunol.*, 1937, **33**, 149.

dermally into rabbits, the infiltration being type-specifically neutralizable by antipneumococcal sera. Subsequent experience has shown that for a given amount of antigen the extent of the neutralizing effect is proportional to the antibody content of the antisera. In the absence of information as to the nature of the active principle it was tentatively called "the irritating substance". Type specific carbohydrates alone do not produce infiltration. On the other hand, since there is type-specificity in neutralization with antisera, it must be concluded that the specific soluble substance determines the specificity of the reaction. Therefore, it seemed possible that the "irritating substance" might be related to the long sought complete antigen of the pneumococci.

Because of the apparent lability of the "irritating substance" toward most manipulative procedures, attempts to obtain further knowledge of its nature by chemical methods were unsuccessful. Recently, however, we have obtained the active principle by sonic disintegration of the pneumococci.

The magnetostriction oscillator used in this work was similar to that previously employed by Chambers and Flosdorf[2] in the sonic extraction of labile antigenic substances from *Strep. hemolyticus* and *Eberthella typhosa*. Suspensions of the bacteria were exposed in lots of about 40 ml to 9000 c.p.s. vibrations of sufficient intensity to cavitate the liquid vigorously. Temperature of the cultures was so controlled by water cooling that it did not rise above 20°C at any time.

Experiments were done with pneumococci of types 1 and 2, grown for 6 hours at 37°, and with formalinized vaccines from 6-hour cultures of pneumococci of Types 1, 2, 3, 7, and 14. All strains used were highly virulent and the cultures and vaccines were strictly Gram-positive. Heavy suspensions containing 20 to 100 billion organisms per cc were used. All types gave essentially the same results and can therefore be reported together. With the oscillator we used, 60 to 75 minutes of vibration were found necessary to destroy all cocci. Progress of the dissolution could be followed on smears made at different times during the treatment. The cocci started to turn Gram-negative after 15 minutes. More and more amorphous detritus appeared until finally almost no cocci remained. Simultaneously the suspensions gradually cleared, assuming the translucence of a typical protein solution. During strong centrifugation the detritus settled down as a whitish sediment, the supernatant

2 Chambers, L. A., and Flosdorf, E. W., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 631.

liquid being transparent but showing a pronounced Tyndall effect.

This supernatant material gave strong precipitation up to 1/20,000 dilution with homologous rabbit and horse antipneumococcal sera. With heterologous antipneumococcal sera there was often some precipitation but only up to dilutions of 1/200 to 1/400. It is not yet possible to make any statement as to the nature of the species specific reaction, but there is no doubt that the material reacts predominantly type-specifically. There was no great loss of activity upon passage of the supernatant liquid through Berkefeld "N" filters, and the resultant colloidal solutions, completely free from formed cellular elements, gave typical type-specifically neutralizable skin reactions in rabbits. It is difficult to estimate the quantities of irritating substances present in the solutions but the reactivity was definitely less in the treated material as compared with the original suspension of cells.

The amorphous sediment remaining in the centrifuge tubes gave no skin reaction whatever.

When the active solutions were frozen their activity remained unimpaired during several weeks of preservation. Some material flocculated out during storage, but it could be removed by centrifuging and was not skin reactive, while the final supernatant solution retained all its reactive qualities.

We tried to evoke antibody formation in rabbits with material of this kind obtained from living pneumococci of Types 1 and 2, and from formalinized vaccines of Types 2, 3, and 7. Altogether 20 rabbits were injected intravenously with increasing doses for 5 weeks. No antibody formation was observed *in vitro*.

We have, therefore, a material which shows one quality more than the S substance, namely skin reactivity, but which does not evoke antibody formation in the rabbit. The faculty of giving skin reactions in the sense defined above is evidently not confined to the complete type-specific antigen of the pneumococcus, but is present at a level of organization intermediate between it and the carbohydrate. These facts are summarized in Table I.

TABLE I.
Stages of Type-specific Pneumococcal Reactivity.

	Reactivity in test tube	Reactivity in skin of rabbits	Antibody formation in rabbits
Specific soluble substance	+	—	—
"Irritating substance"	+	+	—
Whole pneumococcus	+	+	+

The data presented show that intense sonic vibration disintegrates pneumococci into a colloidal solution and an amorphous remainder, and that the solution contains a product which reacts predominantly type-specifically *in vitro* and gives type-specifically neutralizable reaction in the skin of rabbits. It does not elicit antibody formation in these animals.

We are continuing this investigation especially to obtain the active principle in a more purified state. It will be interesting to determine what relation it bears to the "conjugated carbohydrate" recently described by Sevag.[3]

[3] Sevag, M. G., *Science*, 1938, **87**, 304.