osamine bands were eluted, and the eluates were brought to dryness as described above.

**Separation of muramic acid from peptide.** Muramic acid is usually present in smaller amounts than the other two amino sugars, and its rate of movement is sufficiently different that it is easily separated from them in the preliminary run. It has an RF value of 1.10 compared with glucosamine in isopropanol-water (4:1), in n-butanol-pyridine-water (2:2:1), and in isopropanol-n-butanol-water (7:1:2). However, this muramic acid band was found to be heavily contaminated with peptide material. After elution from the preliminary run, it was separated from the peptide by rechromatography with ethyl acetate-pyridine-water (12:5:4) in an ascending system (Fig. 2 and 3). The use of ammonia in the solvent systems should be avoided because of the alkali lability of muramic acid (Strange, Nature 187:30, 1960).

It may be that this method would not be effective in the separation of amino sugars other than glucosamine and galactosamine. However, the success encountered in the present case suggests it may be useful in the isolation of other amino sugars.

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**TYPE-SPECIFIC STREPTOCOCCAL IMMUNIZATION WITH ORAL VACCINE**

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Streptococcal immunity appears to be type-specific, and protective antibodies are directed against the M antigen of the streptococcal cell wall (Lancefield, J. Immunol. 89:307, 1962). Animals have been immunized successfully by parenteral injection of whole bacteria, cell walls, and other streptococcal preparations. Type-specific immunity has been demonstrated by active and passive protection tests and by detection of circulating anti-M antibodies. In man,
type-specific antibodies develop after natural streptococcal infection, but successful primary immunization has not been accomplished because toxicity of available streptococcal antigens allows for the injection of only very small amounts of these.

Since M protein is known to be resistant to hydrochloric acid, it occurred to us that orally administered vaccine might escape complete destruction in the gastrointestinal tract and that a sufficient amount might be absorbed to produce antigenic stimulation.

A highly mouse-virulent encapsulated strain of type 3, group A streptococcus (B 930 obtained from Dr. Lancefield) was grown in Todd-Hewitt broth and then killed in 0.5% phenol. The dead bacteria were washed, dried, and mixed with powdered Purina Laboratory Chow to give a final concentration of approximately 0.3%. A total of 36 female Swiss mice (6 weeks old) were fed this mixture so that each mouse received 10 mg of streptococcal cells per day for a total of 28 days. A control group of 36 mice were given the same amount of Purina Chow without streptococci. All mice were fed plain Purina Chow for an additional week and then were weighed. The weight, which averaged 28 g, was approximately the same for the controls and the mice that were fed streptococci.

Mice from both groups were challenged intraperitoneally with 0.5 ml of varying dilutions of a 3-hr broth culture of strain B 930 (type 3) or a culture of strain S 43 (type 6). All mice were observed for 1 week after challenge. The data in Table 1 show that strain B 930 in dilutions of 10^{-3}, 10^{-4}, and 10^{-7} was lethal for all control mice. A 10^{-8} dilution killed two of five animals. In many previous experiments, this strain of streptococcus was found to have a similar high degree of virulence.

Mice that had been fed B 930 streptococcal vaccine appeared to have developed some immunity to this strain. Although all were killed by 0.5 ml of a 10^{-4} dilution of broth culture, 70% of those given 10^{-4} and 10^{-7} dilutions survived, and all survived an injection of the 10^{-8} dilution. Data showing results of challenge with strain S 43 (type 6) suggest possible induction of slight nonspecific immunity in animals fed B 930 vaccine.

Although the number of animals in each group is relatively small, statistical analysis shows that the difference between the orally immunized animals and the control animals with regard to type-specific immunity is highly significant (P is between 0.007 and 0.01).

Sera obtained from the orally immunized and control animals, when tested by the bactericidal method of Rothbard (J. Exptl. Med. 82:93, 1945), could not be shown to have measurable type-specific antibodies.

The finding of apparent type-specific protection in orally immunized mice in the absence of detectable bactericidal antibodies is consistent with our observation (unpublished data) that parenteral immunization with small amounts of streptococcal vaccine will likewise produce a significant degree of type-specific protection in the absence of measurable amounts of antibody.

If these results can be confirmed with vaccines made from other serological types of group A streptococci, the method of oral immunization may have practical value in man, since animals fed the vaccine seemed to suffer no ill effects.

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