saccharide as that fed was added to the feces of a control rat, and the final precipitates in the tubes for this animal were given an arbitrary value of 10, while the precipitates in the corresponding tubes of the unknown were graded with these as standards. The figures for each animal were averaged and an average of these in turn was calculated. In several experiments, each of 5 or more rats, 75% to 85% of the quantities ingested have been detected in the feces. However, the errors are such as to yield a low value for the quantity present. Proper controls were set up. The averages for each of 7 rats in a typical experiment were, 99, 103, 110, 80, 86, 97 and 43%, giving a final average of 87%.

An experiment in which the soluble specific substance was injected subcutaneously showed that no detectable quantity was eliminated by the bowel. It follows from this that its appearance in the feces after feeding is not the result of absorption and resecretion, either with the bile or otherwise.

It has also been found that Type 2 polysaccharide is eliminated with the feces to the same extent as Type 1.

The recovered polysaccharide of Type 1 pneumococcus from the feces of one set of rats was fed to a new group of animals and these were thereby immunized.

In the case of mice, which unlike rats, have not been found to become more resistant to pneumococcus Type 1, following ingestion of the polysaccharide, the feces contain quantities comparable to those excreted by rats. Appreciable destruction of the carbohydrate thus does not take place in this animal either, and hence cannot be the explanation for the failure to protect mice with this substance.

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A Practical Method for Concentrating Chill Free Pneumococcus Antibodies from Sera Without Use of Salt Precipitations.

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Avery[1] and Felton[2] showed that pneumococcus antibodies were irregularly precipitated from the antisera by simple dilution of distilled water. Banzhaf[3] showed that by fractioning the antisera with

[1] Avery, O. T., *J. Exp. Med.*, 1915, **21**, 133.

[2] Felton, L. D., *Boston M. and S. J.*, 1924, **190**, 819.

[3] Banzhaf, E. J., PROC. SOC. EXP. BIOL. AND MED., 1925, **22**, 329.

sodium sulphate and also with ammonium sulphate the antibodies could be precipitated from the dialyzed globulin on dilution with distilled water. Felton,[4] using the sodium sulphate method to recover the antibodies from the dialyzed globulins, found that at this stage the chill producing substance could be eliminated by dilution. He added sufficient normal sodium sulphate to a point where most of the antibodies were held in solution (the amount depending upon the length of dialysis). He then corrected the reaction to pH 4.8, at which point antibodies are in solution and a considerable amount of inert proteins together with the chill producing substances are precipitated.

It occurred to us that if we diluted antisera to a point where there was a definite precipitate and corrected the reaction to about pH 5, the small amount of antibodies precipitated would become soluble and the inert protein together with the chill producing substances would precipitate. This we found did occur. We obtained the same results without dilution by dialyzing the antisera practically free from salts and then adding sufficient sodium chloride or sodium sulphate to have a normality of about 1/20 and correcting the reaction to pH 5-5.1.

These methods were reported by Dr. William H. Park at the Harben Lectures in London October 10th, 1930, and are as follows: The cold antisera containing 0.4% trikresol or 0.5% phenol are diluted with cold distilled water, one part serum to 3 parts of distilled water, a definite precipitate occurs. When corrected to pH 5-5.1 using gamma dinitrophenol 0.025% solution as indicator, precipitated material will almost completely dissolve, followed immediately by a reprecipitation of inert protein together with the chill producing substances. It is then kept at from 5°C. to 10°C. for 4 hours, then filtered clear through paper pulp and adjusted to pH 6.8 using para nitrophenol 0.1% solution as indicator. One-tenth per cent trikresol or phenol is added at this time and allowed to remain in a cool place over night as usually a fibrin like substance will be noticed next day. If present it is again passed through paper pulp and trial tubes set up to determine the amount of distilled water necessary to precipitate the antibodies. This rarely requires more than an equal volume of distilled water. The antibodies are allowed to settle over night in a cold room. The supernatant is then decanted and the precipitate placed in cold centrifuge cups and rapidly centrifuged. The centrifuged precipitate is roughly estimated in cubic centimeters and a like amount of one per cent sodium chloride containing 0.8%

[4] Felton, L. D., *J. Inf. Dis.*, 1925, **37**, 199, 309.

phenol is added and the precipitate dissolved. The volume measured and diluted with 0.5% salt solution containing 0.4% phenol to one tenth of the original serum used. The solution is kept in a cool place for at least 24 hours for any possible clouding or precipitation that may occur. It is then passed through paper pulp to clarify and one percent sodium chloride added to bring the salt content up to 1.5%. It is then filtered (Berkefeld) and tested for sterility and potency.

The method of concentrating the antisera without the primary dilution is as follows: Antiserum containing 0.4% trikresol is dialyzed in running tap water for 3 days and 0.2% of trikresol is added and sufficient sodium chloride to have the salt concentration 1/20 normal. It is then chilled to 5°C. after which the reaction is corrected to pH 5-5.1. The method described in the foregoing is now followed to conclusion.

As this work was finished the November *Journal of Immunology* was received. In it Doctor Kenneth Goodner reports on experiments on the concentration of antipneumococcus and antimeningococcic horse sera. His work and ours is somewhat similar. He stresses cold temperature and the necessity for determining by trial the amount of distilled water to be added to 5 cc. of the antisera to obtain the first cloud and then adding 8 cc. more. This he states will precipitate all the antibodies. His work indicates a purer antibody can be obtained than we report in this paper. He states, however, nothing about removing the chill producing substances which we believe to be in his product.

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A Practical Method for Concentrating Chill Free Pneumococcus Antibodies from Plasma Without Use of Salt Precipitations.

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Antipneumococcus plasma containing 0.4% trikresol is dialyzed in running tap water for 4 days. This will remove sufficient salts for the method to be described. The plasma and precipitate consisting mostly of fibrinogen, euglobulin and antibodies are removed from the dialyzing bags, sufficient sodium chloride or sodium sul-