

from the plausibility of the assumption that there is one. Until the presence of such a vitamin is established in natural infants' diets in cases where rickets is prevented and a deficiency in diets of cases where rickets occurs it will be safer to approach the subject with an open mind. The rickets-curing substances in cod liver oil and in egg yolk might just as well be looked upon as therapeutic agents, possibly internal secretions, which will prevent or cure rickets.

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On the nature of pneumococcus antigen.

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Various extracts of killed pneumococci have been studied for the purpose of determining their immunizing power and their freedom from toxic products. A non-toxic pneumococcus antigen of high potency can be made by suspending the sediment from a centrifuged pneumococcus broth culture in saline and digesting the pneumococci with 0.2 per cent. trypsin for 24 to 48 hours. The undigested portion is centrifuged off and the metaproteins in the supernatant fluid precipitated with acid. After filtration the filtrate is thrown into 7 volumes of 95 per cent. alcohol, the resulting precipitate filtered off and the alcoholic solution evaporated in vacuum. The residue in the flask is taken up in saline and made up to the original volume of the saline suspension. This solution contains the immunizing antigen of the pneumococcus. Mice which had received three subcutaneous injections of this antigen were protected against a hundred thousand lethal doses of a pneumococcus broth culture, injected intraperitoneally.

Human volunteers were injected subcutaneously with the alcohol soluble fractions of pneumococcus Type I, II, and III.

Three doses were given, the total corresponding to 50 to 75 billion of killed pneumococci. Practically no local, and absolutely no general reaction was observed after any of the injections. Following the injection of antigen protective substances against the three fixed types of pneumococcus could be demonstrated in the serum of the volunteers.

The pneumococcus antigen is not adsorbed by Lloyd's reagent, and does not diffuse through a collodion membrane.

Saline suspensions of pneumococcus Type I which had been stored in the refrigerator for several months were shown to contain a large amount of the antigen in the supernatant fluid. Mice were injected subcutaneously with two or three doses of the supernatant fluid which protected them against a hundred thousand lethal doses of a broth culture of pneumococcus Type I, injected intraperitoneally. Some of the immunizing antigen is retained by the killed pneumococci.

A chemical analysis of the supernatant fluid from old pneumococcus vaccine gave the following result: Total nitrogen, 13 mg. per 100 c.c., non-protein nitrogen, 12.9 mg. per 100 c.c., of which 8 mg. were in the form of amino acid nitrogen. Phosphate present. Reaction P_H 7.8. Biuret, Millon, Xanthoproteic and Hopkins-Cole reaction were all negative. Precipitation tests with heat and acid, sulphosalicylic acid, potassium ferrocyanide and acetic acid were all negative. A slight turbidity is produced by 9 volumes of 95 per cent. alcohol, trichloroacetic acid, conc. hydrochloric and nitric acid. The precipitate produced by 9 volumes of alcohol was found to be chiefly phosphates.

The filtrate from old pneumococcus vaccine gives clear cut precipitin reaction with homologous antiserum in antigen dilutions up to 1:120.

The immunizing antigen of the pneumococcus and the precipitinogen appear to be two distinct entities, as we have encountered some pneumococcus antigen solutions which fail to give precipitin reaction with homologous antiserum, but produce immunity in mice when injected subcutaneously.