phosphorus rather than in the ratio of Ca:P. 3.3 gm. of KH_2PO_4 was added to the McCollum diet, rendering the phosphorus equivalent to the content of calcium in the McCollum diet, and the ratio of Ca:P was made about 4:4. On this ration the calcium in the blood fell as with the previous rations. We next made up a diet similar to the McCollum ration with the exception that 2.36% less of $CaCO_3$ was incorporated; the standard amount of $CaCO_3$ is 3%. By this means a ratio of Ca:P of 1:1 was brought about and the ration did not contain any more phosphorus than the rickets-producing McCollum ration (0.302 gm.). Nevertheless the calcium concentration in the blood fell after a period of 2 to 4 days, and the phosphorus rose.

It was evident that the tetany did not result from giving a ration in which phosphorus was relatively high compared to calcium nor in which it was greater in absolute amount than in the McCollum ration. The sudden fall of calcium in the blood with the accompanying symptoms seemed rather to be the reaction to the sudden change of the Ca:P ratio as compared to the high ratio of (4:1) which characterized the rickets-producing diet. This sudden change, associated with healing of the rickets, evidently so disturbed the Ca:P balance in the body that the calcium concentration in the blood could no longer maintain its level and hyper-irritability of the nervous system followed.

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Fate of Orally Administered Specific Polysaccharide of Pneumococcus.

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The author reported that, following the ingestion of Type I pneumococcus polysaccharide, white rats possess an increased resistance to the homologous organism.[1] It consequently became of interest

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[1] ROSS, Victor, PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 658.

to learn something concerning the fate of this material when thus administered. Furthermore, the promptness with which immunity appears[2] after feeding the pneumococcus, suggested the oral administration of dead pneumococci in the early stages of human pneumonia as a therapeutic measure. It therefore became of importance to know whether the contained soluble specific substance enters the circulation. If it does, such a procedure would seem to be contraindicated, since it has been shown that the specific polysaccharide inhibits the pneumococcidal action of a normal serum-leucocyte mixture.[3]

Since the carbohydrate confers immunity when taken by mouth it seems reasonable to suppose that it is absorbed from the intestinal tract. Its polysaccharide nature would suggest, on the other hand, that its absorption as such is improbable.

The blood serum was examined for the polysaccharide of Type I pneumococcus following oral administration. Interfering sediments obtained when some rat sera are mixed with horse serum caused considerable difficulty in deciding whether a given precipitate was indicative of the presence of minute amounts of the polysaccharide or not. Preliminary incubation of the animals' sera and of the immune horse serum largely avoided such interference. By adding known amounts of Type I polysaccharide to rat serum, it was found that the presence of 1 part in a million in serum can be detected with a fair degree of confidence in the result. In the experiments in which quantities up to 6 mg. were fed, we have been unable to find definite evidence that the polysaccharide is present in a concentration of 1 part per million in serum. It seems that if it enters the circulation at all, the polysaccharide is present in a smaller concentration than this. Insufficient examinations have been done to say definitely whether or not it can be found in the urine. More work will be done with blood and urine. However, it is of interest to note that a large proportion can be found in the feces.

Briefly, the method employed was as follows: The polysaccharide was extracted from the feces by means of HCl, the solution made alkaline, and the sediment removed by centrifugation. Following neutralization of the supernatant fluid, serial dilutions were made so that the dilution in the final tube was equal to approximately 1 part in 6 millions after adding the serum and on the assumption that all that was fed was present in the feces. Incubation took place in an ice box for 48 hours. The same amount of poly-

[2] Ross, Victor, *J. Exp. Med.*, 1930, **51**, 585.

[3] Sia, R. H. P., *J. Exp. Med.*, 1926, **43**, 633.

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.