tions differ so greatly from those of water that insulin is also being studied in other solvents, such as aqueous solutions of serum proteins in which it is appreciably soluble. The far lower solubility of insulin in water than in propylene glycol, a type of behavior characteristic also of zein, indicates that insulin may be considered a crystalline animal prolamine.

After repeatedly electrodialyzing crystalline zinc insulin in the preparation of the amorphous material used in the above experiments, it occurred to us to recrystallize the amorphous insulin with radioactive zinc. For this purpose a sample of zinc containing the radioactive Zn^{65} isotope, of half-life 250 days, was employed.[8] The electrodialyzed insulin was crystallized with radioactive zinc from acetate buffers, and amorphous commercial insulin was crystallized from phosphate and recrystallized from acetate buffers, following the method of Scott.[9] The radioactivity of the dried crystals was determined with a Lauritsen type quartz fiber electroscope. The radiation was filtered through 2.35 mm of aluminum in order to record only the gamma rays, which are much less affected than beta particles by self-absorption in samples of varying bulk. Comparison was made in each case with a standard sample of zinc sulfide or zinc ammonium phosphate prepared from the same sample of zinc. Under the conditions of electrodialysis given above, no more than 0.31 per cent. radioactive zinc was detected in our final products of crystalline insulin. Scott reports that crystalline zinc insulin contains 0.52 per cent. zinc. In order to determine whether our lower content of radioactive zinc depended upon incomplete removal of zinc by electrodialysis under the conditions employed, a commercial amorphous preparation[10] believed to contain not more than 0.03 per cent. Zn was crystallized with radioactive zinc. These crystals were estimated to contain 0.36 per cent. radioactive zinc.

Further experiments with radioactive zinc will be undertaken in order to determine the maximum content of radioactive zinc that can be introduced into crystalline insulin, and the conditions under which the zinc can be removed from insulin by dialysis or electrodialysis in acid or neutral solutions, or by interchange with blood and tissue proteins. Our experiments thus far indicate that none of the radioactive zinc introduced into insulin is removed at neutral reactions by electrodialysis or prolonged dialysis, but that at the isoelectric point of insulin the radioactive zinc is quantitatively lost to normal horse serum. When, however, insulin containing two atoms of radioactive zinc per mole was separated from serum at neutral reaction, by precipitation with the protamine, salmin,[2] the resulting precipitate retained 0.14 per cent. radioactive zinc, corresponding approximately to a mono-radioactive zinc insulin protaminate.

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[8] Livingood and Seaborg, *Phys. Rev.*, 55: 457, 1939.

[9] Scott, *Biochem. Jour.*, 28: 1592, 1934; Scott and Fisher, *Biochem. Jour.*, 29: 1048, 1935.

[10] We are indebted to Dr. George B. Walden, of Eli Lilly and Company, for this material.

TYPE-SPECIFIC ANTIBODY PRODUCTION WITH LIVING PNEUMOCOCCI IN THE RABBIT

IT is generally held that only virulent strains are satisfactory as antigens for preparing antipneumococcus sera and that it is at least doubtful that immunization with living pneumococci is of value.[1] Griffith[2] writes that "living attenuated culture is useless for preparation of protective serum, and, as is known, this is due to the absence of type-specific antigen in such strains. This essential complex is liable to disintegration in living cultures, both prior to and subsequent to inoculation, with the result that non-type-specific antibodies are produced in the serum."

It was found recently[3] that a strain of attenuated tubercle bacilli, *Bacillus* Calmette-Guerin, induces antibody formation against virulent bovine tubercle bacilli more readily than heat-killed suspension of virulent micro-organisms. This observation stimulated us to determine whether living attenuated pneumococci are more effective immunizing agents than killed suspensions of virulent strains. Pneumococci of low virulence agglutinable by the type-specific antibody can be procured by repeated subcultures on blood bouillon and omission of the customary passage through mice. The first series of experiments were carried out with a Type III strain because sera against this type are of low potency and mouse-virulent strains are of relatively low virulence for rabbits. The lethal dose of the strain used is more than 20 cc for adult rabbits.

The centrifugalized sediment of five hours pepton bouillon cultures was suspended in 1 cc of bouillon per dose of "vaccine" and inoculated intravenously into rabbits weighing from two to three and one-half kilograms. Most of the rabbits received the sediment of 5 cc of culture on three successive days of the week for four weeks; later the doses were increased to 5, 5 and 10 cc. Agglutinin and precipitin titers of the sera of rabbits so immunized are considerably higher than those of animals immunized with killed suspension of cultures passed through mice frequently. Type-specific antibody nitrogen determination made by Dr. Kenneth

[11] Society of Fellows, Harvard University.

[1] B. White, E. S. Robinson and L. A. Barnes, "The Biology of Pneumococcus," The Commonwealth Fund, New York, 1938; H. Zinsser, J. F. Enders and L. D. Fothergill, "Immunity Principles and Application in Medicine and Public Health," The Macmillan Company, New York, 1939.

[2] F. Griffith, "A System of Bacteriology," Vol. II, His Majesty's Stationery Office, London, 1929.

[3] J. Freund, J. Casals and E. P. Hosmer, *Proc. Soc. Exp. Biol. and Med.*, 37: 509, 1937; J. Freund and E. L. Opie, *Jour. Exp. Med.*, 68: 273, 1938.

Goodner of the Rockefeller Institute have shown in a small group of rabbits that the majority of the sera contained from one to two mg antibody nitrogen per cc, and one exceptional serum contained approximately 6.4 mg.

Similar experiments are being carried out with pneumococci of Type I. The results indicate that the method described above is applicable to production of Type I antipneumococcic serum.

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THE SIGNIFICANCE OF THE AMINO ACIDS IN CANINE NUTRITION[1]

IN previous publications from this laboratory[2,3] it has been shown that only ten of the twenty-two amino acids known to exist in proteins are *indispensable* dietary components. These are tryptophane, lysine, histidine, phenylalanine, leucine, isoleucine, threonine, methionine, valine and arginine. With the exception of arginine, the removal of any one of these compounds from the food leads to a profound nutritive failure, accompanied by a rapid decline in weight, loss of appetite and eventual death. On the other hand, the exclusion of arginine from the ration is followed by much less pronounced effects. The subjects continue to gain, but at a suboptimal rate. This is accounted for by the fact that arginine, in contrast to the other members of the indispensable group, can be manufactured by the cells,[4] but at a speed which does not quite keep pace with the demands of *normal* growth. The twelve remaining amino acids are dispensable in the sense that they can be synthesized in adequate amounts out of materials ordinarily available in the organism.

In arriving at the above conclusions young rats served as the experimental animals. Consequently, it does not follow necessarily that the findings are applicable to other species. Indeed, a *quantitative* difference in the arginine requirement has already been recorded in the literature. The growth of chicks is said to be accelerated by the addition of this amino acid to the ration, even when the latter contains 18 per cent. of casein[5] (equivalent to a dietary arginine content of approximately 0.68 per cent.). This amount of casein furnishes more than three times the quantity of arginine necessary for the growing rat.[2] It becomes of importance, therefore, to ascertain whether the rat is singularly proficient in the synthesis of amino acids, or whether other mammals also manifest the ability to thrive on relatively simple mixtures.

The nutritive rôle of the individual amino acids has now been established for the adult dog. This was undertaken as a preliminary to extensive investigations on the maintenance of nitrogen equilibrium in various species by oral and intravenous alimentation. The program has advanced sufficiently to warrant a brief report at this time. The experimental findings and the details of the technique will be presented elsewhere.

Adult females were used throughout. As a rule, they were first brought into nitrogen equilibrium upon a casein diet, and were then transferred to a similar ration in which mixtures of highly purified amino acids served as the sole sources of nitrogen, except for traces introduced unavoidably as contaminants of the vitamin B concentrates. The urine samples were collected by catheterization at intervals of twenty-four hours, and the feces were divided into periods of seven days by the administration of carmine capsules.

The first dog received a ration containing the ten amino acids found previously to be indispensable for the growing rat. She promptly manifested a slight positive nitrogen balance, and continued to do so, with a moderate gain in weight, for the duration of the test (4 weeks). Obviously, *the amino acids which are dispensable for the growing rat are also dispensable for the adult dog.* At the beginning of the fifth week, arginine was dropped from the diet. The change was without influence upon either the body weight or the nitrogen balance of the subject. Thus, *for the adult dog arginine is not a necessary dietary component.* This finding was not unexpected. Nearly two years ago we[3] predicted that arginine would prove to be dispensable for full-grown animals. The experiment was discontinued at the end of the eighth week, at which time the dog was in perfect nutritive condition.

Three dogs were employed in determining the physiological importance of the other amino acids of the indispensable group. Invariably, the removal from the food of any one of these compounds was followed by a pronounced negative nitrogen balance. Furthermore, the restoration of the missing amino acid to the diet uniformly resulted in a positive nitrogen balance. These data demonstrate that *the qualitative amino acid needs of the dog are identical with those of the rat.* The fact that two widely different species require for their well-being the same components of the protein molecule, increases the probability that other mammals, including man, may manifest like responses.

In the experiments herein described the amino acid

[1] The researches upon which this report is based were supported in large measure by a grant from the Rockefeller Foundation.

[2] W. C. Rose, SCIENCE, 86: 298, 1937.

[3] W. C. Rose, *Physiol. Rev.*, 18: 109, 1938.

[4] C. W. Scull and W. C. Rose, *Jour. Biol. Chem.*, 89: 109, 1930.

[5] A. Arnold, O. L. Kline, C. A. Elvehjem and E. B. Hart, *Jour. Biol. Chem.*, 116: 699, 1936; A. A. Klose, E. L. R. Stokstad and H. J. Almquist, *Jour. Biol. Chem.*, 123: 691, 1938.