glucose. Solutions of sodium and potassium salts having the same pH were non-inhibiting.

Grove and Grove[7, 8] called attention to the importance of ammonia in saliva several years ago, but the significance of their observations has not been grasped, perhaps because large quantities of ammonia nitrogen were not detected in the saliva of caries immunes. Small concentrations of ammonia formed more or less continuously on the vulnerable surfaces of the teeth may confer a natural protection against dental caries. The above authors as well as Stephan[9] have reported lowering caries incidence in many cases by using ammonia and urea mouth rinses.

The question arises as to what is present in the salivas of caries-free individuals that provides the ammonia. Early studies by us indicate that the source is not alone urea or mucin but the presence of various amino acids. Twenty-three amino acids alone and in various combinations have been checked for the possibility of ammonia production from them by the enzyme systems of the bacteria previously found in the salivary cultures.

Preliminary experiments have revealed that caries-immune individuals have enzyme systems capable of converting at least six amino acids into ammonia nitrogen. The six are arginin, alanine, aspartic acid, asparagin, glutamic acid, isoleucine and serine. Certain combinations of these amino acids proved much more effective. A wide variance of enzyme systems have been found in salivas from persons whose teeth are actively decaying. However, there seems to be in most instances an absence in the saliva of caries-active patients of a system that will convert glutamic acid to ammonia.

Microbiologic assays are now underway to determine the amino acid content of caries-immune and caries-active salivas. Our study to date suggests that caries immunity is based on the production of minute but continuous amounts of ammonia in the bacterial plaque resident on the tooth surface. The pabulum from which the ammonia is derived is apparently a small group of amino acids. These are present in the mouth as a result of the type of diet and body metabolism.

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THE PREPARATION OF HIGHLY PURIFIED PR8 INFLUENZA VIRUS FROM INFECTED MOUSE LUNGS[1]

ONE of the most convincing lines of evidence in establishing that tobacco mosaic virus activity is a specific property of a characteristic high molecular weight nucleoprotein was the demonstration that preparations of the virus protein from different species of plants, some of which were widely removed from the tobacco family, possessed essentially the same chemical, physical and biological properties.[2] This approach has not heretofore been used for viruses affecting animals because essentially pure preparations of virus from different animal hosts have not been available. Recently, highly purified preparations of PR8 influenza virus have been obtained from the allantoic fluid of infected chick embryos.[3,4] These preparations contain particles about 100 mμ in diameter possessing characteristic chemical, physical and biological properties.[3-9] It appeared, therefore, that if one could isolate a comparable product from another host, an approach similar to that used for tobacco mosaic virus could be made and thus provide important data on the nature of influenza virus produced in different hosts. The possibilities of comparison were enhanced by the fact that influenza virus appears to possess at least two major forms of biological activity, namely, virus activity and red cell agglutinating capacity.

An attempt was made therefore to obtain purified preparations of PR8 influenza virus from suspensions of infected mouse lungs. When methods of centrifugation alone were employed, such as had been used previously by other workers,[10,11] it was discovered that preparations were obtained which had low chick-cell agglutinating (CCA) activities and which obvi-

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and The Rockefeller Institute for Medical Research.
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ously contained large amounts of non-viral lung material. The procedure which was finally adopted and which led to a highly active product was one which combined the methods of adsorption on and elution from chicken red cells[12,13] and differential centrifugation. Four week-old mice from the colony of the Department of Animal and Plant Pathology of the institute were inoculated in a special chamber with an extremely fine spray of a 1 per cent. suspension of infected mouse lung[14] in 0.1 M phosphate buffer at pH 7. After about 72 hours, the mice were placed under deep ether anesthesia and their lungs were perfused with 0.85 per cent. saline and removed. The lungs were ground in 9 times their weight of 0.1 M phosphate buffer at pH 7 in a Waring Blendor, and the resulting suspension was angled in a low-speed centrifuge to remove gross particles. Such clarified 10 per cent. suspensions were accumulated and stored at about −70° over the course of 3 months until somewhat more than a liter had been obtained. The frozen material was then thawed, mixed thoroughly, and centrifuged to remove insoluble matter. The supernatant liquid possessed 113 standard CCA units[15] per ml and 50 per cent. infectivity endpoints in mice[16] and in chick embryos[17] at $10^{-6.6}$ ml and $10^{-8.7}$ ml, respectively. To 1,380 ml of cold clarified suspension were added 14 ml of chicken red cells. After standing overnight, the red cells were removed by centrifugation. The virus was then eluted from the red cells by incubation at 37° for 90 minutes in 200 ml of 0.1 M phosphate buffer at pH 7 and the resulting product was freed from blood elements by 2 cycles of differential centrifugation. This process yielded a product possessing more than 130 times the CCA activity of the starting material on a mg of nitrogen basis, but the value, 11,200, was still low compared to the CCA activity of chick embryo virus. Hence the adsorption and elution process was repeated followed by 2 additional cycles of differential centrifugation. The final product possessed about 28,000 CCA units per mg of nitrogen, a value which compares favorably with that of PR8 virus preparations obtained from allantoic fluid and which is about 17 times that of the best preparations obtained from mouse lungs by centrifugation alone. In so far as determined, the physical, chemical and biological properties of the purified material obtained from infected mouse lungs are indistinguishable from those of purified preparations of PR8 virus obtained from allantoic fluid. The preparation was highly infectious for both mice and embryos and gave 50 per cent. infectivity endpoints at 10^{-11} and 10^{-13} grams of nitrogen in mice and in chick embryos, respectively. In contrast to the bulk of material in preparations obtained from mouse lungs by centrifugation alone, the product obtained by the combined methods was soluble in 0.1 M phosphate buffer at pH 7 and gave a good boundary in the analytical ultracentrifuge. The sedimentation constant corrected for the viscosity of the preparation was 683S.[8] Electron micrographs revealed approximately spherical particles which were indistinguishable from those of the virus produced in chick embryos. The material was isoelectric at pH 5.4, contained about 10 per cent. of nitrogen and 7 per cent. of carbohydrate and precipitated strongly with antiserum to PR8 virus grown in chick embryos. As will be described in greater detail elsewhere, it is believed that a preparation of PR8 influenza virus has been obtained from infected mouse lungs that possesses essentially the same physical, chemical and biological properties as the virus preparations obtained from the allantoic fluid of chick embryos infected with PR8 influenza virus. The facts that the 2 hosts from which highly active preparations of virus have now been obtained are of such decidedly different nature and the sites of infection are so dissimilar, add to the significance which the findings have regarding the nature of viruses which infect animals and the use of heterologous sources of virus in the production of prophylactic vaccines.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

STERILITY TEST FOR PENICILLIN EMPLOYING CYSTEINE FOR INACTIVATION

CAVALLITO and Bailey[1] recently noted that cysteine was capable of readily inactivating a number of antibiotics including penicillin. This fact might be used to advantage in testing for the sterility of penicillin preparations.

For a preliminary test of the method, a penicillin solution containing 1,830 International Units and 10

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