SOCIETIES AND MEETINGS

THE SEISMOLOGICAL SOCIETY OF AMERICA—EASTERN SECTION

THE Eastern Section of the Seismological Society of America held its fourteenth annual meeting in Freeman Hall on the campus of Fordham University, New York City, June 9 and 10, 1939. The Reverend Joseph Lynch, S.J., well-known seismologist and head of the department of physics, welcomed the guests and members of the section.

The sessions were officially opened on Friday morning at 9:15 by the chairman, H. E. McComb, of the U. S. Coast and Geodetic Survey. To allow time for more informal discussion and a tour of some of the scientific exhibits of the World's Fair, a departure was made from the usual procedure. Two days are customarily devoted to the presentation of committee reports, election of officers and the reading of papers. This year the seismologists continued in session until 7:15 in the evening, completing the scheduled program and stopping only long enough at 1:30 to enjoy a buffet luncheon as guests of the university. Because of the warm weather the lunch was served in one of the basement laboratories, where suction flasks and pyrex beakers provided the necessary "scientific" atmosphere.

Reassembled again at 2:30 P.M., the delegates resumed with presentation of papers and reports. Animated discussion attended the reading of the report of the committees on microseisms and amateur seismology and the paper of A. C. Chick, Providence, R. I., on "Trends in Earthquake Insurance since 1933." Other papers of special interest should be mentioned: "The Nature and Origin of Microseisms," by J. Emilio Ramirez, S.J., of Bogotá, Colombia; "The Problem of Earth Deformation," by M. King Hubbert, Columbia University, and a report by A. G. Ingalls, of the editorial staff of the *Scientific American,* on "Amateur Seismology." The formal meeting was terminated by the reports of the resolutions and nominating committees and the election of officers for the ensuing year. The unanimous choice was as follows: *Chairman,* A. C. Ruge, of the Department of Civil Engineering, Massachusetts Institute of Technology; *Vice-Chairman,* A. J. Westland, S.J., Department of Physics, Spring Hill College; *Secretary,* W. A. Lynch, Department of Physics, Fordham University; *Treasurer,* H. Landsberg, Geophysical Laboratory, Pennsylvania State College; *Fifth Member of the Executive Committee,* H. E. McComb, of the U. S. Coast and Geodetic Survey.

At 10 o'clock Saturday, June 10, practically the entire group assembled again for a three-hour informal discussion of current seismological problems. Topics of timely import were, a new speed control for seismograph drums, demonstrated by A. C. Ruge; the necessity of putting all our seismographs to work; the new instruments and equipment at cooperative stations of the U. S. Coast and Geodetic Survey; the need for more sensitive instruments in the Gulf Coast States.

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SPECIAL ARTICLES

STUDIES IN THE PHYSICAL CHEMISTRY OF INSULIN[1]

I. THE SOLUBILITY AND DIELECTRIC PROPERTIES OF INSULIN AND ITS CRYSTALLIZATION WITH RADIOACTIVE ZINC

INSULIN, which can be crystallized in a state of high purity, and possesses unusual chemical stability, as well as specific physiological properties, is a very suitable protein for quantitative physical chemical studies. This paper gives preliminary results of a series of investigations in progress in these laboratories upon the physical chemical properties of insulin and its interaction with ions and dipolar ions.

Crystalline zinc insulin[2] was dissolved in sufficient N/10 hydrochloric acid to bring the final pH to 2.5, electrodialyzed against N/300 hydrochloric acid in a Brintzinger type electrodialyzer for four hours, and then freed from acid by continuing the electrodialysis against distilled water for twenty hours. The protein precipitated in amorphous form; the supernatant solution had a conductivity of about 3×10^{-6} ohm^{-1} cm^{-1} and was chloride-free. The insulin was washed with conductivity water.

Measurements of solubility in water and glycine solutions were carried out at 5° by rotating portions of amorphous insulin prepared in the above manner with successive aliquots of solvent. After each equilibration, lasting from two to eight days, the suspension was allowed to settle, and the supernatant solution filtered through sintered glass. Analysis was performed by precipitating the insulin with trichloracetic acid, washing free of solvent with dilute trichloracetic acid solution, digesting the protein and determining the nitrogen colorimetrically with Nessler's reagent.

[1] From the Department of Physical Chemistry, Harvard Medical School, and the Jefferson Physical Laboratory, Harvard University.

[2] Furnished through the kindness of Eli Lilly and Company.

The solubility in water of insulin thus prepared never exceeded 0.027 grams per liter, but diminished to approximately a third of this value upon repeated equilibration with water. Although denaturation of the insulin with prolonged washing is not precluded, pharmacological activity[3] had not diminished in a sample which had been washed fifteen days, the solubility of which had decreased to 0.014 grams per liter. The average solubilities, when a constant level had been reached, are designated by the hollow circles in Fig. 1, initial solubilities by circles with crosses.

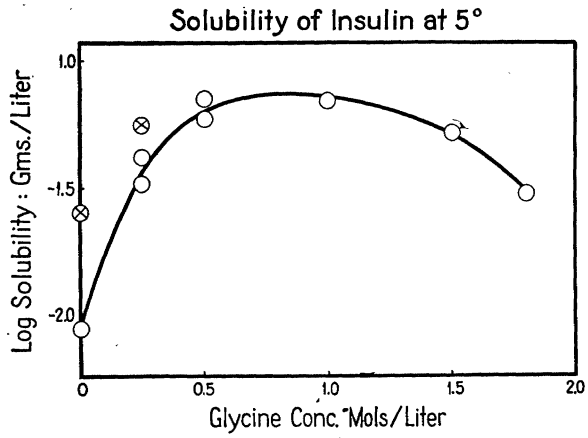

FIG. 1

Solubility of insulin in solutions containing 0.5 M glycine or more did not diminish upon re-equilibration with solvent, but remained constant for as many as nine equilibrations over a period of thirty days. These solutions saturated with insulin always had a pH close to 6.1, characteristic of pure glycine, rather than of the isoelectric point of the insulin. The average solubility was 0.065 grams per liter in 0.5 M glycine, 0.069 grams per liter in 1.0 M, 0.052 grams per liter in 1.5 M and 0.030 grams per liter in 1.8 M glycine. The occurrence of maximum solubility at a glycine concentration not greater than 1.0 M indicates a large "salting-out" effect. Indeed, the solubility in 1.8 M glycine is smaller than in 0.5 M glycine, whereas for hemoglobin[4] the "salting-out" effect reduces solubility at this concentration only to approximately that in 1.0 M glycine. The observed solvent action of glycine upon insulin, if corrected for the "salting-out" effect by an equation comparable to that previously employed for hemoglobin ([5], equation 5), would suggest interaction between these two dipolar ions somewhat greater than observed for hemoglobin. Lactoglobin has thus far been studied[6] only at glycine concentrations lower than half molal, where no "salting-out" effect could be detected and the observed interaction was far greater than with insulin or hemoglobin.

The rôle of the dipole moments in the interactions of dipolar ions has previously been stressed.[5] Taking the dipole moment of glycine as 15, that of hemoglobin[7] has been estimated as 500 and of lactoglobulin[6] as 700, on the basis of measurements of dielectric constant increments of 0.33 and 1.4, respectively, per gram per liter.

The concentration of insulin prepared as above, even in 1.0 M glycine, is too low to contribute appreciably to the dielectric constant of the solution. Accordingly electrolyte-free solutions of insulin in quantities sufficient to study its dielectric properties were prepared in other solvents. Solutions of 1.9 grams per liter in propylene glycol, 3.2 grams per liter in propylene glycol containing 10.2 per cent. and 3.6 grams per liter in propylene glycol containing 20 per cent. of water were studied. The total dielectric increments from high to low frequencies, $\Delta\varepsilon_t$, were 0.26, 0.29 and 0.38 per gram per liter, respectively, in these three solvents. The dispersion curves are given in Fig. 2, where the

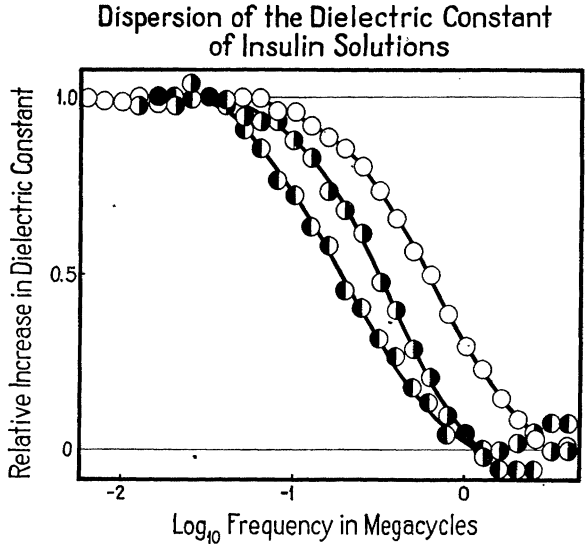

FIG. 2. Dispersion of the dielectric constant of insulin at 25° C. in ◐ propylene glycol (viscosity relative to water, $\eta_r = 47.5$), ◑ propylene glycol with 10.2% water ($\eta_r = 28$), ○ propylene glycol with 20% water ($\eta_r = 17$).

relative increase in dielectric constant is plotted against the logarithm of the frequency. The mean relaxation times in the three solvents, when divided by the viscosities relative to water, agreed within 10 per cent., the average value being 1.7×10^{-8} seconds.

The viscosities and dielectric constants of these solu-

[3] As judged by mouse test, for which we are indebted to Dr. W. T. Salter, of the Department of Medicine, Harvard University.

[4] M. M. Richards, *Jour. Biol. Chem.*, 122: 727, 1938.

[5] Cohn, McMeekin, Ferry and Blanchard, *Jour. Phys. Chem.*, 43: 1, 1939.

[6] Ferry, Cohn, Oncley and Blanchard, *Jour. Biol. Chem.*, Proceedings (1939).

[7] J. L. Oncley, *Jour. Amer. Chem. Soc.*, 60: 1115, 1938.

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.