ATTEMPTS AT PURIFICATION OF A MURINE STRAIN OF HUMAN POLIOMYELITIS VIRUS[1]

THE SK strain of human poliomyelitis virus adapted to rodents by Jungeblut and Sanders[2] offers convenient material for physico-chemical study because of its high virulence in mice and its relative stability. Preliminary information on the physical nature of the murine strain of this virus is presented here with an outline of the method used for its purification.

Infected mouse brains, kept frozen in the refrigerator, are ground with 9 volumes of saline, containing 0.1 per cent. glycine. Glycine is now being added routinely to all virus preparations, since this substance was found to increase considerably the stability of the virus in high dilutions. The 10 per cent. brain suspension is clarified by low-speed centrifugation and the supernatant is removed and vigorously shaken with one quarter volume of ether. This material is recentrifuged for one half hour in the large tubes of the Swedish angle centrifuge. The fats collect at the top, leaving a clear fluid below which has the same potency as the original brain suspension (*i.e.*, 10^7 to 10^8 M.L.D. per 0.03 cc intracerebral inoculum in mice) and a protein content of about 0.2 per cent. To this fluid is added slowly one quarter volume of M acetic acid-Na acetate buffer, pH 4.6. The precipitate which forms is centrifuged down and the supernatant discarded; the precipitate is washed in saline-acetate buffer, recentrifuged and thoroughly resuspended in a volume of 0.12 M Na_2HPO_4 equal to one tenth that of the original solution. The insoluble precipitate is centrifuged off and discarded, whereas the supernatant is further clarified by addition of three tenth volume of saturated ammonium sulfate solution. The new precipitate which forms is removed by centrifugation, leaving a clear supernatant which is freed from ammonium sulfate by dialysis. The virus titer of this solution is 10^8 to 10^9 M.L.D., with a protein concentration of ca. 0.1 per cent. This operation, therefore, concentrates the virus nearly ten-fold without significant loss and removes at the same time about 95 per cent. of the non-active material. The solution just described is stable and may be kept in the ice-box for weeks without significant loss in titer.

Attempts to extract the virus from this solution by chemical means—such as precipitation with ammonium sulfate, NaH_2PO_4, acetic acid or adsorption on either tricalcium phosphate or aluminum hydroxide—in general, resulted in appreciable inactivation of the virus. These attempts were therefore abandoned. It may also be mentioned in passing that in the course of this work peculiar granular precipitates were occasionally observed, which may have been similar to the material recently described by Racker.[3] They were not associated, however, with particularly high infectivity, and therefore their nature was not further investigated; the possibility of their being virus was later dismissed when it appeared that there was too little virus present to yield such precipitates.

Extraction of the virus from the same solution by ultracentrifugation gave more satisfactory results. The following experiment may be cited as an example. Fifty cubic centimeters of the solution (protein concentration ca. 0.1 per cent.) were centrifuged at an average velocity of 600/r.p.s. for 38/minutes. The tubes were decanted and allowed to drain, and the hardly visible sediments were resuspended in a total volume of 1 cc of buffer. The fluid thus obtained, after discarding some coarse impurities, was bluish and slightly opalescent and had a protein content of the order of 0.01 per cent. (as estimated by precipitation with sulfosalicylic acid) which showed that only a small percentage of the solutes present consisted of high molecular weight material. The titer of the solution before centrifugation being 10^8 M.L.D., after centrifugation the titer of the supernatant was 10^6 and that of the resuspended sediment 10^9, indicating that practically all the virus had been sedimented. However, since the theoretical titer of the resuspended material should have reached $10^{9.7}$, it is probable that the virus was not entirely recovered, or that some inactivation had occurred. In view of the fact that the 50 cc which were centrifuged had been prepared from 44 gm of mouse brain, the yield appears to have been less than 1 mgm virus per 100 gm of infected mouse brain, representing about one part in 10,000 of the total brain proteins, an exceedingly small amount indeed.

Further attempts were made to estimate the sedimentation constant of the virus in this same solution. For this purpose the material was centrifuged in the sectorial cell of the ultracentrifuge, or in capillary tubes placed horizontally in the Swedish centrifuge, and the supernatant was sampled after runs of varying length and velocity. A value of the order of

[1] Aided by grants from the Warner Institute for Therapeutic Research and the Philip Hanson Hiss, Jr., Memorial Fund.

[2] C. W. Jungeblut and M. Sanders, *Proc. Soc. Exp. Biol. and Med.*, 44: 375, 1940; *Jour. Exp. Med.*, 72: 407, 1940; *Jour. Am. Med. Assoc.*, 116: 2136, 1941; C. W. Jungeblut, M. Sanders and R. R. Feiner, *Jour. Exp. Med.*, 75: 611, 1942; C. W. Jungeblut, R. R. Feiner and M. Sanders, *Jour. Exp. Med.*, 76: 31, 1942.

[3] E. Racker, SCIENCE, 96: 364, 1942.

200 *S* was obtained, which corresponds to a molecular weight of 10 million or more, depending on molecular shape. These experiments also revealed that, after complete sedimentation, the virus could not, in general, be recovered without some loss in titer, due presumably to inactivation or to mechanical difficulty in completely resuspending a very small sediment.

Comparison of the observations recorded here for SK murine poliomyelitis virus with the results obtained by Gard and Pedersen,[4] who worked with Theiler's virus of spontaneous mouse encephalomyelitis, suggests that the two viruses are not significantly different in the physical sense. Thus, Theiler's virus was found to have a sedimentation constant of 160; a molecular weight of 52 million and molecular dimensions of 640 mμ for the long diameter and 14 mμ for the short diameter were estimated from sedimentation and optical diffusion measurements; these data were obtained with a fraction which represented 0.5 mgm out of 100 gm of infected mouse brain, and which carried practically all the original infectivity. The preliminary results reported here for SK murine poliomyelitis virus are therefore in fair agreement with those of the Swedish workers. For human poliomyelitis virus obtained from monkey cords, a slightly lower sedimentation constant of 62 has been recently reported.[5] The activity of the viruses belonging to the poliomyelitis group, therefore, regardless of the strain of virus considered, appears to be always associated with material of very heavy molecular weight. On the other hand, these viruses are able to pass through membranes of small porosity, and particle diameters of about 10 mμ have been obtained from the filtration of all three strains.[6,7,8] The apparent contradiction between sedimentation and filtration measurements is not completely resolved if one makes the permissible assumption that all three strains consist of long thread-like molecules, since the lengthwise progression of such molecules through collodion membranes of low permeability does not seem quite plausible. It is probably more rational, therefore, to suppose that the rapid sedimentation of these viruses is due to the fact that they are present mostly as large aggregates of elementary particles, conceivably in linear association, which are able to break up, to some extent, into smaller active units. This view would be supported by analytical measurements of the diffusion of Theiler's virus.[9]

[4] S. Gard and K. O. Pedersen, SCIENCE, 94: 493, 1941.
[5] H. S. Loring and C. E. Schwerdt, *Jour. Exp. Med.*, 75: 395, 1942.
[6] W. J. Elford, I. A. Galloway and J. R. Perdrau, *Jour. Path. and Bact.*, 40: 135, 1935.
[7] M. Theiler and S. Gard, *Jour. Exp. Med.*, 72: 49, 1940.
[8] M. Sanders and C. W. Jungeblut, *Jour. Exp. Med.*, 75: 631, 1942.
[9] J. Bourdillon, *Jour. Gen. Physiol.*, 25: 263, 1941.

The essential purity of the materials obtained by Gard and Pedersen or by us can not be assessed as long as none of the criteria usually employed for chemical purity can be applied. It may be remarked, however, that the protein present in one lethal dose of the ultracentrifuged preparation described above must have weighed about 3×10^{-15} gm, which is equivalent to 180 molecules with a molecular weight of 10 million. If one assumes that an appreciable proportion of these molecules were in an inactive state, only a comparatively small number of active molecules could have been present to constitute one lethal dose for mice. This in turn may be taken as a suggestion that "pure" virus represented a substantial fraction of the material isolated.

JAQUES BOURDILLON
DAN H. MOORE
DEPARTMENTS OF BACTERIOLOGY AND ANATOMY,
COLLEGE OF PHYSICIANS AND SURGEONS,
COLUMBIA UNIVERSITY

DIHYDROXY-STEARIC ACID AND VITAMIN K DEFICIENCY

THE fat depots of animals show relatively high acetyl values, indicating the presence of considerable hydroxy-fatty acids. These acids, therefore, are normal constituents of omnivorous dietaries.

It has been reported that butterfat is superior nutritionally to vegetable oils.[1] The appearance of this report prompted the suggestion that possibly this superiority was due to the hydroxy-acids in butterfat, since butterfat is relatively rich in these acids and their presence in animal tissues may be presumed to indicate a nutritional significance. Later it was reported[2] that the active factor in butter is a long-chain saturated fatty acid.

To test our thesis a synthetic fat containing a proportionally large amount of dihydroxy-stearic acid was prepared and fed in the dietary of albino rats. The data on the nutritive values of hydroxy-acids will be presented later but we wish at this time to report interesting abnormalities observed during a part of this investigation.

A control group of 25 weanling rats was put on a diet containing 18 per cent. casein (Labco), 53 per cent. sucrose, 25 per cent. hydrogenated fat (Spry) and 4 per cent. mineral salts.[3] Each rat received a daily supplement of 60 I.U. vitamin A, 6 I.U. vitamin D, 0.02 mg thiamine, 0.025 mg riboflavin, 0.10 mg

[1] E. J. Schantz, C. A. Elvehjem and E. B. Hart, *Jour. Dairy Sci.*, 23: 181, 1940.
[2] E. J. Schantz, R. K. Boutwell, C. A. Elvehjem and E. B. Hart, *Jour. Dairy Sci.*, 23: 1205, 1940; R. K. Boutwell, R. P. Geyer, C. A. Elvehjem and E. B. Hart, *Jour. Dairy Sci.*, 24: 1027, 1941.
[3] R. B. Hubbell, L. B. Mendel and A. J. Wakeman, *Jour. Nutrition*, 14: 273, 1937.