# STUDIES ON THE PURIFICATION OF POLIOMYELITIS VIRUS

## I. Yields and Activity of Preparations Obtained by Differential Centrifugation*

By HUBERT S. LORING, Ph.D., and C. E. SCHWERDT

*(From the Department of Chemistry, Stanford University, California)*

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The application of high speed differential centrifugation to the purification of poliomyelitis virus has been a logical step since Stanley and Wyckoff (1) showed that relatively small and unstable plant viruses could be isolated as homogeneous materials by this method. The sedimentation of poliomyelitis virus in an ultracentrifuge was first demonstrated by Schultz and Raffel (2), and more recently Clark, Rasmussen, and White (3) obtained sediments which showed an irregular but in some instances high virus activity. While it has been shown that high speed centrifugation resulted in a sedimentation of the virus, evidence has not been provided that other macromolecular materials associated with normal tissue were not also sedimented along with the virus under these conditions. In view of the work of several investigators (4–6) on the concentration of macromolecular materials from various normal animal tissues, such constituents might very well be present together with virus in the high molecular weight fractions obtained from poliomyelitis-infected tissues.

This paper presents the results of experiments on the application of differential centrifugation to the extracts of glycerolated or frozen, infected medullae-cords from *rhesus* monkeys and to similar extracts of glycerolated or frozen, normal medullae-cords. The results show that when extracts of infected as well as normal cords, that have been stored in glycerol, are subjected to high speed centrifugation, appreciable yields of high molecular weight substances are obtained. However, when normal tissue that had been frozen and stored for appreciable lengths of time was used, no significant amount of high molecular weight, nitrogen-containing compounds was recovered. Frozen infected tissue, on the contrary, yielded small but significant amounts of high molecular weight material possessing both a high and uniform virus activity.

### EXPERIMENTAL

Purification of the virus by differential centrifugation requires (1) the preparation of large quantities of infectious extract free from tissue fragments

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and lipoidal material, (2) the separation of the virus from soluble constituents of low molecular weight, and (3) the separation of the virus from high molecular weight substances which are thrown down with the virus in the ultracentrifuge. The first step was accomplished by suspending the infected cords in buffer by means of a Waring blendor, by centrifuging the tissue suspension at 3800 R.P.M. in a large angle centrifuge, by extracting the supernatant liquid with ether, and then by filtering the aqueous extracts through celite. The virus was sedimented, and aggregated colloidal material was removed by centrifugation in an air-driven centrifuge of the improved Pickels' design[1] (7). The virus used was the M. V. strain mixed with a strain provided by Dr. Charles Armstrong in 1936 and carried in monkeys since then by Dr. E. W. Schultz. The details of the procedure which was applied both to infected and normal tissues are as follows:

The spinal cord and medulla from each animal was removed aseptically and either dropped into a 1-1 glycerol-0.1 M phosphate buffer mixture at pH 7 and stored in a refrigerator or placed in a large test tube (25 × 200 mm.) and stored in a freezing chamber at −10°C. About 100 gm. of the pooled glycerolated tissue, drained free of the glycerol-phosphate solution, or the same amount of the frozen tissue, and 200 ml. of buffer cooled to 0°C. were mixed in the blendor for 3 minutes. During this time, the temperature of the mixture increased to about 15°C., and it was necessary to cool the container in ice before mixing was continued. In this way the suspension was mixed for a total of about 15 minutes. It was then transferred to 100 ml. centrifuge bottles and centrifuged at 3800 R.P.M. in an angle centrifuge in a room maintained at 2°C. until a relatively clear supernatant fluid was obtained. The time necessary varied with the solvent used. About 1½ hours were necesssary when Ringer's solution was used and about 3 to 4 hours when water or 0.05 M phosphate or 0.01 M borate buffer was used. The supernatant fluid was pipetted off, and the residue again transferred to the blendor and extracted as before with an additional 100 ml. of ice-cold solvent for about 3 minutes. This suspension was centrifuged in the angle centrifuge, and the supernatant fluid removed as before. In this way a third, fourth, and in many cases a fifth extract was prepared.

The extracts, which at this stage were milky in appearance, were combined and extracted in a separatory funnel with one-half a volume of ether. An emulsion was obtained which was broken in the angle centrifuge into an ether layer, a middle layer of semisolid gelatinous material, and a clear aqueous bottom portion. The aqueous layer was removed, and the semisolid material re-extracted once with buffer. The amount of this semisolid material varied greatly with the buffer used in preparing the original extract. It was decreased to a thin skin-like layer in each centrifuge tube when Ringer's or physiological salt solution was used but was as thick as a centimeter when the tissue was extracted with water or dilute buffer. The extracts were now almost water clear with the exception of a few particles of solid which were removed by filtration through a thin layer of a mixture of approximately equal

[1] The authors are indebted to Dr. E. G. Pickels for providing photographic copies of scale drawings of the driving mechanism.

parts of Hyflo and Standard supercel. The filtrate from either infected or normal cords was a clear liquid usually slightly pink in color due to the presence of small amounts of hemoglobin. That from poliomyelitic tissues was infectious in about 100 per cent of the monkeys injected intracerebrally with 1 ml. of a 1 to 1000 dilution based on the weight of cords extracted.

A volume of the clear filtrate equal to 112 ml., in eight stainless steel tubes was centrifuged at 600 R.P.S. for 1½ hours in a dowmetal head similar in design to that described by Wyckoff and Lagsdin (8). The head was cooled to 0°C. at the beginning of a run and warmed up to about 10°C. after 1½ hours. The average centrifugal force was about 79,000 × gravity. After a centrifugation run the supernatant liquid was carefully pipetted off and replaced with an equal amount of the clear extract, which was in turn centrifuged at 600 R.P.S. In this way the high molecular weight

TABLE I

*Examples of Analyses of Supernatant Liquids after Successive Sedimentations of High Molecular Weight Constituents at 600 R.P.S.*

| Experiment No. | Amount and type of starting material | Total nitrogen in | | | | | |
|---|---|---|---|---|---|---|---|
| | | Original extract | Supernatant liquids from successive centrifugations | | | | |
| | | | First | Second | Third | Fourth | Fifth |
| | | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* |
| 6 | 100 gm. glycerolated virus cords | | 269 | 0.63 | 0.11 | | |
| 19 | 88 gm. frozen normal cords | 245 | 229 | 3.4 | 0.80 | 0.30 | 0.26 |
| 20 | 85 gm. frozen virus cords | 272 | 268 | 1.74 | 0.36 | 0.14 | 0.035 |
| 22 | 44 gm. frozen virus cords | 187 | 180 | 1.67 | 0.47 | 0.19 | 0.14 |
| 23 | 103 gm. frozen virus cords | 205 | 196 | 1.23 | 0.16 | 0.031 | 0.011 |

With the exception of Experiment 22, the pooled tissues were obtained from *rhesus* monkeys. In Experiment 22, the tissues were obtained from *cynomolgus* monkeys. The solvents used were as follows: in Experiment 6, 0.05 M phosphate at pH 7.5; in Experiments 19 and 22, 0.01 M borate at pH 7.8; and in Experiments 20 and 23 Ringer's solution. Nitrogen determined by method of Levy and Palmer (9).

materials in the extract from 100 gm. of tissue and in some cases from 200 gm. was concentrated into eight tubes. The sediments were combined and resuspended in about 28 ml. of solvent. Aggregated colloidal particles were removed from this solution in the air-driven centrifuge by running it at a speed of 500 R.P.S. for a few minutes. The supernatant fluid and the wash liquid from the sediment obtained after a similar run up and down were then subjected to a second sedimentation run at 600 R.P.S. for 1½ hours. The supernatant liquid was pipetted off, the sediment resuspended in buffer, and aggregated material again removed as before.

Analyses of the supernatant liquids from each successive sedimentation showed that the amount of unsedimentable nitrogen decreased and was insignificant after the fourth sedimentation. Most preparations were, therefore, subjected to four centrifugal cycles of purification before they were considered free from low molecular weight constituents. Examples of the amounts of nitrogen present in successive supernatant liquids from extracts of infected and normal tissues when phosphate, borate, or Ringer's solution was used are shown in Table I.

The sediment obtained after four sedimentations was dissolved in a small volume of solvent, the solution was centrifuged at slow speed to remove any insoluble residue, and an aliquot was analysed for total nitrogen by the method of Levy and Palmer (9). Aseptic technique was used throughout the purification procedures and the temperature was kept as near 0°C. as possible. The time required for the preparation of the extracts and for the purification of the high molecular weight constituent from 100 gm. of tissue averaged about 10 days.

### *Recovery of Virus Activity after Ultracentrifugation*

To determine the efficiency of the above described method in sedimenting the virus and to obtain a rough measure of the loss of virus in the discarded supernatant liquids, a sample of infectious filtrate was subjected to three centrifugal cycles of purification. This involved sedimentation, removal of the supernatant liquid and re-solution of the sedimented material in fresh solvent. The activity of the sediment was compared with that of a similar quantity of filtrate, which had been treated in the same way with the exception that the sediment was redissolved each time in the original supernatant liquid. The results found in three experiments are shown in Table II.

*Activity Tests.*—In all cases in which a preparation was tested for virus activity, 1 ml. of the dilution or concentration tested was injected intracerebrally into *rhesus* monkeys under ether anesthesia. The dilutions were prepared immediately before inoculation in the same buffer used for the extraction of the infected tissues. After injection the animals were observed daily for tremors and the onset of a flaccid paralysis. Those which developed paralysis were sacrificed within a day or two after the paralysis had become extensive. In a few cases in which animals died during the incubation period without showing paralysis, histological examinations were made of the medulla and cord. If typical lesions were found, the test was considered positive. Animals which failed to develop poliomyelitis within 3 weeks after injection were counted negative, and were used again in later experiments. These monkeys almost without exception finally came down with poliomyelitis.

It can be seen that the amount of virus activity present in the sediment obtained after one or three centrifugal cycles of purification was approximately the same as that of the original extract or of the control in which the sediment was redissolved each time in the original supernatant liquid. The amount of virus aggregated because of packing in the centrifuge or lost in the discarded supernatant liquids was not sufficiently great, therefore, to be detected by these infectivity measurements. Several experiments on the activity of the supernatant liquids obtained after a run at 600 R.P.S. gave results which led to the same conclusion. The supernatant liquid was infectious in only a small percentage of the animals injected with doses of 1 ml. Before ultracentrifugation, 1 ml. of the clarified extracts was usually infectious at a dilution of about 1:120.

*Yields of High Molecular Weight Materials from Glycerolated and Frozen Infected and Normal Tissues*

The yields of sedimentable nitrogen obtained from poliomyelitis-infected and normal cords when subjected to the above described procedures are

TABLE II

*Recovery of Poliomyelitis Virus Activity after Centrifugation at 600 R.P.S. for 1½ Hours*

| | Dilutions (based on weight of tissue extracted) | | | |
|---|---|---|---|---|
| | 1–500 | 1–1000 | 1–2000 | 1–4000 |
| 1. Original extract. | | | | |
| Experiment 1 | ++* | ++ | +− | +− |
| 2. Original extract centrifuged and sediment resuspended in supernatant liquid. | | | | |
| Experiment 1 | ++ | +− | −− | −− |
| " 2 | ++ | +− | ++ | +− |
| 3. Original extract centrifuged, supernatant liquid discarded, and sediment resuspended in volume of buffer equal to that of supernatant liquid. | | | | |
| Experiment 1 | ++ | +− | ++ | −− |
| " 2 | | +− | +− | −− |
| 4. Once-sedimented virus treated two additional times as in 2. | | | | |
| Experiment 1 | ++ | +− | ++ | ++ |
| " 2 | ++ | +− | −− | +− |
| " 3 | ++ | +− | ++ | |
| 5. Once-sedimented virus treated two additional times as in 3. | | | | |
| Experiment 1 | ++ | +− | ++ | +− |
| " 2 | ++ | +− | +− | +− |
| " 3 | ++ | ++ | ++ | |

In Experiments 1 and 2 Waring blendor extracts before ether extraction were used. In Experiment 3 the extract was further clarified by ether extraction, etc. In Experiments 1, 2, and 3 the buffer consisted of 0.01 molar borate at pH 7.8, distilled water, and Ringer's solution without glucose respectively.

* Each + sign indicates that the monkey developed poliomyelitis. Each − sign indicates that the monkey failed to develop poliomyelitis within 3 weeks, but later either proved susceptible or died from other causes.

shown in Table III. Data are included for tissues that had either been stored in glycerol at 5°C. or kept in the frozen state at −10°C. To provide a measure of the length of time the tissues were stored in each experiment, the average age of the pool is given. This was obtained by dividing the total length of time all the cords in a pool were stored by the number present. The number of

cords, in most experiments, and the dates they were removed and the extracts prepared are as follows:

*Experiment 7.*—Aug. 15, 1938, to Nov. 18, 1939. Extracted Apr. 22, 1940; Experiment 11. May 15 to June 2. Extracted June 19, 1940; Experiment 16. Aug. 9 to Oct. 14; Extracted Oct. 23, 1940; Experiment 17. June 1 to July 8. Extracted Nov. 9, 1940; Experiment 19. One each on Feb. 7 and 9, three on Mar. 19, and five on Mar. 20. Extracted Mar. 21, 1941; Experiment 20. One each on Dec. 4, 5, 13, 27, 28, and 30, 1940, and Mar. 7, 1941, and two each on Jan. 6 and Mar. 6. Extracted Apr. 4, 1941; Experiment 21. One each on Nov. 17, 28, and 29, and three each on Nov. 16 and 20, 1940. Extracted Apr. 15, 1941; Experiment 22. Three on Apr

TABLE III

*Yields of High Molecular Weight Constituents from Infected and Normal Tissues*

| Type of tissue | Method of storage | Experiment No.* | Average length of time tissue was stored | Time required for purification | Amount of tissue ground | No. of sedimentations | Yield (nitrogen) | Yield nitrogen per 100 gm. tissue |
|---|---|---|---|---|---|---|---|---|
| | | | *days* | *days* | *gm.* | | *mg.* | *mg.* |
| Infected | In 50 per cent glycerol-phosphate at 5°C. | 7 | 386‡ | 30 | 107 | 4 | 0.97 | 0.91 |
| | | 11 | 26 | 20 | 195 | 3 | 0.95 | 0.49 |
| | | 16 | 42 | 8 | 92 | 3 | 2.1 | 2.3 |
| | | 17 | 140 | 77 | 85 | 4 | 0.57 | 0.67 |
| | | 27 | 283 | 11 | 209 | 3 | 0.98 | 0.47 |
| Infected | In stoppered tubes without solvent at −10°C. | 20 | 81 | 6 | 85 | 5 | 0.15 | 0.18 |
| | | 21 | 145 | 8 | 70 | 3 | <0.01 | <0.01 |
| | | 22 | 7 | 8 | 44 | 5 | 0.51 | 1.16 |
| | | 23 | 85 | 9 | 103 | 5 | 0.078 | 0.076 |
| | | 24 | 105 | 10 | 170 | 3 | 0.12 | 0.071 |
| | | 26 | 30 | 12 | 186 | 3 | 0.19 | 0.10 |
| | | 28 | 9 | 8 | 170 | 3 | 1.0 | 0.59 |
| | | 29 | 13 | 17 | 73 | 4 | 0.16 | 0.22 |
| | | 30 | 19 | 8 | 93 | 3 | 0.39 | 0.42 |
| Normal | In 50 per cent glycerol-phosphate at 5°C. | 31 | 54 | 13 | 57 | 3 | 0.22 | 0.39 |
| Normal | In stoppered tubes without solvent at −10°C. | 19 | 4 | 10 | 70 | 5 | 2.74 | 3.91 |
| | | 25 | 54 | 10 | 111 | 3 | 0.04 | 0.036 |
| | | 32 | 34 | 4 | 112 | 3 | 0.18 | 0.16 |

* Experiments 19, 20, 22, and 23 are the same experiments referred to in Table I. The solvents used in the other experiments were as follows: 7, 11, and 16, 0.05 M phosphate at pH 7.5; 17, 0.01 M sodium borate at pH 7.8; 21, Ringer's solution; in all other experiments Ringer's solution without glucose.

‡ The cords in this pool were collected in connection with earlier studies carried out by Professor E. W. Schultz.

21 and five each on Apr. 20 and 22. Extracted Apr. 28, 1941; Experiment 23. Five on Nov. 13, 1940, one each on Mar. 8, 9, 11, and Apr. 17, two on Apr. 19, and four on Apr. 20. Extracted May 14, 1941; Experiment 24. Nine on Nov. 14 and three on Nov. 15, 1940, one each on May 8, 10, 12, 16, and 18, three on May 14, two each on May 15 and 17. Extracted May 29, 1941; Experiment 25. One each on Mar. 25, Apr. 16, 22, and June 10, two on May 24, and four on July 15. Extracted July 21, 1941; Experiment 26. May 21 to July 22. Extracted July 25, 1941; Experiment 27. One each on Oct. 15, 16, 24, 25, 26, Nov. 8, 9, and 11, two each on Oct. 21 and Nov. 4, and four on Nov. 12, 1940. Extracted Aug. 11, 1941; Experiment 28. One each on July 23, Aug. 6, 22, 23, and 26, two each on Aug. 24 and 25, three on Aug. 8 and five each on Aug. 14 and 16. Extracted Aug. 27, 1941; Experiment 29. Five each on Aug. 26 and 27. Extracted Sept. 9, 1941; Experiment 30. One each on Aug. 26 and Sept. 19, three on Sept. 20, and six on Sept. 22. Extracted Oct. 8, 1941; Experiment 31. One each on June 24, July 18, 19, and Oct. 8, two on Oct. 7. Extracted Oct. 21, 1941; Experiment 32. One each on Aug. 14 and Nov. 4, two each on Aug. 4, Oct. 9 and 28, three on Oct. 10, and four on Oct. 30. Extracted Nov. 11, 1941.

Appreciable amounts of high molecular weight materials were obtained from both glycerolated normal and infected tissues regardless of the length of time the tissues were stored. The results with frozen tissues, in general, show a striking decrease in the amounts of high molecular weight substances present. It is evident, however, that the yield varies, depending on the period of storage. In Experiments 22 and 28, in which the cords had been stored for only a short time, relatively large quantities of high molecular weight materials were obtained. In contrast to these, in Experiments 21, 23, and 24, in which the tissues had been kept for from 2 to 5 months on the average, only small yields of sedimentable nitrogen were isolated. As shown in Experiments 19, 25, and 32, similar results were obtained with normal tissue stored at $-10°C$. While the number of the latter experiments is not sufficiently large to lead to a final conclusion, the data suggest that such storage has a more pronounced effect on the high molecular weight constituent present in the normal than in the infected cords.

*Virus Activity of Sediments from Glycerolated and Frozen Infected Tissue*

The virus activities of the sediments from glycerolated and frozen infected cords were determined by finding the minimum quantity in terms of nitrogen, which would produce typical poliomyelitis when injected intracerebrally into normal *rhesus* monkeys. The results found for eleven different preparations are shown in Table IV. It can be seen that with the exception of Experiment 22, infections were regularly obtained with $5 \times 10^{-9}$ gm. of nitrogen of the virus sediment in a volume of 1 ml., when the infected tissue had been frozen before extraction. In most cases some infection was also obtained with $5 \times 10^{-10}$ gm. The regular infective dose when glycerolated tissue was used was $5 \times 10^{-8}$ to $5 \times 10^{-7}$ gm., and only about 30 per cent of the animals tested with $5 \times 10^{-9}$

gm. developed poliomyelitis. In so far as it is possible to compare these results, therefore, the use of frozen tissue resulted in material about ten times as active as that obtained from glycerolated tissue. The activity found in Experiment 22 can be explained by the fact that in this case the cords had been stored for an average of only 7 days, and the sedimentable nitrogen probably consisted of the normal component as well as of virus. This explanation would also account for the relatively large yield of the high molecular substances found

TABLE IV

*Activities of Individual Preparations from Glycerolated and Frozen Tissues*

| Experiment No.* | Method of storage | Average length of time stored | Concentration injected (gm. nitrogen per ml.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $5 \times 10^{-6}$ | $5 \times 10^{-7}$ | $5 \times 10^{-8}$ | $5 \times 10^{-9}$ | $2.5 \times 10^{-9}$ | $1 \times 10^{-9}$ | $5 \times 10^{-10}$ | $5 \times 10^{-11}$ |
| | | *days* | | | | | | | | |
| 6 | In 50 per cent | 345 | | | | ++−−‡ | −−−− | | | |
| 11 | glycerol- | 26 | | +− | +−− | −− | | | | |
| 16 | phosphate | 42 | +++ | ++− | ++− | +−− | | | | |
| 17 | at 5°C. | 140 | +++ | +++ | ++− | +−− | | | | |
| 20 | | 81 | +++ | +++ | +++ | +++ | | | +−− | −−− |
| 21 | | 145 | | | + | + | | | − | |
| 22 | In stoppered | 7 | | +++ | ++ | ++− | | | −−− | |
| 23 | tubes with- | 85 | | ++ | ++ | ++ | ++ | +− | +− | |
| 24 | out solvent | 105 | | | ++ | ++ | | | −− | |
| 26 | at −10°C. | 30 | | +++ | +++ | ++ | | −−− | +−− | |
| 30 | | 19 | | | ++ | ++ | | | ++ | |

* The experiment numbers refer to the same experiments listed in Table III. In Experiment 20 the tests at $5 \times 10^{-11}$ and $5 \times 10^{-10}$ gm. nitrogen per ml. were made when the sample was 27 days old. In Experiment 21 the dilutions were made assuming a nitrogen concentration of 0.01 mg. per ml. In Experiment 24, the activity tests were made after the sample had stood at about 5°C. for 67 days.

‡ See footnote, Table II.

in this experiment. The constituent from normal cords failed to produce symptoms of disease when doses corresponding to $5 \times 10^{-5}$ gm. of nitrogen were injected intracerebrally.

### *General Properties of the High Molecular Weight Constituents from Infected and Normal Tissues*

The products obtained after three or four sedimentations in the ultracentrifuge from either infected or normal tissues consisted of small, amber-colored, transparent pellets which dissolved completely in water or buffer solutions. In the case of glycerolated cords in which relatively large yields of high molec-

ular weight materials were found, the solutions were in most cases opalescent by reflected light and slightly yellow by transmitted light. The products obtained from frozen cord, regardless of whether they were infected or normal, were appreciably yellow but failed to show the characteristic opalescent appearance by reflected light. The virus concentrates, in two instances, were examined for streaming birefringence by allowing the solutions to flow from a

TABLE V

*Stability of Purified Virus Preparations When Stored for Different Lengths of Time in Different Solvents*

| Experiment No. | Length of time stored | Solvent used | Concentration injected (gm. nitrogen per ml.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $1.5 \times 10^{-6}$ | $5 \times 10^{-7}$ | $2.5 \times 10^{-7}$ | $5 \times 10^{-8}$ | $10^{-8}$ | $5 \times 10^{-9}$ | $2.5 \times 10^{-9}$ | $5 \times 10^{-10}$ |
| | *days* | | | | | | | | | |
| 6 | 1 | 0.05 molar phosphate at pH 7.5 | | | | | | ++−−* | −−−− | |
| | 26 | | | | | | +−− | +−− | | |
| | 51 | | | | −− | +− | +− | | | |
| | 88 | | +++ | + | | | | | | |
| 16 | 5 | 0.01 molar borate at pH 7.8 | +++ | ++− | | ++− | | +−− | | |
| | 21 | | +++− | − | | +−− | | −−− | | |
| | 25 | | +++ | ++− | | −−− | | −− | | |
| | 50 | | ++− | +++ | | −−− | | −−− | | |
| 20 | 1 | Ringer's solution pH 8 | +++ | +++ | | +++ | | +++ | | |
| | 27 | | | +++ | | +++ | | ++− | | +−− |
| | 95 | | | ++++ | | +++ | | ++− | | −−− |

The sample obtained in Experiment 6 was prepared from glycerolated infected tissue with 0.05 molar phosphate buffer as the solvent. The samples in Experiment 16 and 20 are the same as those described in Table III. The three samples at concentrations of 0.1, 2.1, and 0.15 mg. nitrogen per ml. respectively were stored in stoppered tubes at about 5°C.

* See footnote, Table II.

pipette held between crossed polaroid plates. In neither case was there evidence of double refraction of flow.

The purified virus preparations give the Millon's test for protein. The nitrogen in one of the earlier, less active virus samples and that from a normal sample, obtained in both cases from glycerolated material, was completely precipitated by trichloroacetic acid. Several samples from normal and infected cords were examined for phosphorus (10) and carbohydrate (11), and in all cases these constituents were found present. The nitrogen to phosphorus ratio for the normal constituents in the two preparations was 4.5 to 1, and for two virus samples it was 3.7 and 5.3 to 1. The carbohydrate (as glucose)

to nitrogen ratios for the same preparations were 1.1 and 1.3 for the normal samples and 1.2 and 0.3 for the virus samples. However, because the analyses were carried out on extremely small samples, the experimental error was large, and the values are not greatly significant except to demonstrate that both carbohydrate and phosphorus were present.

On the addition of one-half volume of saturated ammonium sulfate to solutions of either the virus-containing sediment (Experiment 30) or the normal constituent (Experiment 32), the solutions became turbid. On allowing them to stand overnight in the refrigerator, a yellow amorphous precipitate separated in both cases. That from the infected cord proved partly insoluble in water or in dilute phosphate buffer at pH 7. The precipitate from normal tissues dissolved completely in Ringer's solution, but its solubility was not determined in the above mentioned solvents.

The purified virus is relatively stable at about 5°C. when dissolved in Ringer's solution. Table V shows the results of activity tests made on three preparations over a period of about 3 months. Some loss of activity probably took place after the solutions had stood for about 25 days, but even after 95 days in the case of the most active preparation (Experiment 20) there was sufficient activity remaining to infect the three animals tested at a concentration of $5 \times 10^{-8}$ gm. of nitrogen. The purified preparations do not retain their activity when dried from Ringer's solution in the frozen state and over phosphorus pentoxide at 40°C. One such preparation, for example, which was 100 per cent infective at a concentration of $5 \times 10^{-9}$ gm. of nitrogen before it was dried, failed to infect two monkeys at $1.3 \times 10^{-5}$ gm. after it was dried.

## DISCUSSION

The experiments on extracts of normal spinal cords and medullae demonstrate that there is a high molecular weight constituent present and that it is sedimented in the ultracentrifuge under the conditions used to sediment the virus. Appreciable yields of this component were obtained either from glycerolated normal or infected tissues or from tissues that had been stored without glycerol at −10°C. for a few days. Storage of normal tissue at −10°C. for longer periods of time resulted in a decrease of this constituent, and after several weeks the amount present became almost negligible. Storage of infected tissue at −10°C. likewise resulted in a decrease in the yield of sedimentable nitrogen, but small amounts of material were still obtained after the tissues had been stored for several months. The specific activity of the sediments under the latter conditions was about ten times that of the preparations obtained either from glycerolated or fresh, infected tissues. It appears, therefore, that the decrease in the yield of high molecular weight substances takes place at the expense of the normal constituent rather than of virus. The experiments on the recovery of virus activity after three ultracentrifugal

cycles of purification provide evidence that the amount of virus, which had become irreversibly aggregated after ultracentrifugation, was not appreciable.

The high and relatively uniform specific activity of the virus preparations obtained from cords that had been stored for several weeks suggests that they may consist of relatively pure virus. A more detailed study of the minimum period of storage necessary to eliminate the high molecular weight constituent completely from normal tissue and of the effect of storage on total virus activity is in progress. These experiments should provide additional information regarding the optimal conditions for obtaining the virus. Some direct evidence for the homogeneity of the purified preparations has been found from studies in the McBain transparent ultracentrifuge (12). In collaboration with Dr. Frances Wright, the homogeneity and sedimentation rate of several preparations were determined. The results, which will be presented in full in a later paper, show that relatively homogeneous preparations were obtained in experiments in which the infected tissues had been stored for several months. The average value for the sedimentation constant $S_W^{20°}$, was $62 \times 10^{-13}$. It is of interest that this value is different from that of $S^{20°}$, $160-170 \times 10^{-13}$ reported recently for the mouse encephalomyelitis virus by Gard and Pedersen (13). If further work definitely establishes the identity of the $62 \times 10^{-13}$ component with the poliomyelitis virus, it would appear that the two viruses differ not only in immunological properties as shown by Theiler (14) but in sedimentation rate as well.

## SUMMARY

Results of experiments on the preparation of high molecular weight constituents from normal and poliomyelitis-infected medullae-cords are presented. Relatively large yields were obtained from glycerolated normal or infected tissues or from tissues that had been stored at $-10°$C. for a few days. When the frozen tissues were stored for several weeks, the amount of sedimentable nitrogen isolated from the normal cords decreased and became almost negligible. Under these same conditions small but definite amounts of a high molecular weight material were isolated from the infectious extracts. This material regularly produced poliomyelitis when 1 ml. containing $5 \times 10^{-9}$ gm. of nitrogen was injected intracerebrally into *rhesus* monkeys. The purified virus sediment contains nitrogen, phosphorus, and carbohydrate, it gives the Millon's test for protein, and is precipitated by one-third saturation with ammonium sulfate. Results of ultracentrifugal analyses show that a relatively homogeneous component with $S_W^{20°} = 62 \times 10^{-13}$ is present.

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