hour and then fractionally distilled at atmospheric pressure to give 8.43 g. (97% yield) of N,N-dimethylacetamide, b.p. 163–165°,[18] $n^{25}D$ 1.4234, and a pot-residue of non-volatile resinous material.

We wish to acknowledge the technical assistance of Messrs. Charles Anderson and Kenneth Hutton.

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[CONTRIBUTION FROM THE VIRUS LABORATORY, UNIVERSITY OF CALIFORNIA]

Physical Chemical Studies on Rabbit Papilloma Virus[1]

BY H. K. SCHACHMAN

It has been suggested by Beard and co-workers that rabbit papilloma virus varies in physical properties from preparation to preparation and that there are natural differences in the virus isolated at different times from different source material. In the light of this interpretation of the experimental data several preparations of rabbit papilloma virus were examined in an ultracentrifuge, Tiselius apparatus and viscometer. An impurity not previously reported was observed in sedimentation and electrophoresis patterns. The high intrinsic viscosities observed were attributed to this impurity. As in the studies of Beard and co-workers the sedimentation constant of the principal component varied from preparation to preparation. These variations were explained on the basis of different viscosities of the preparations due to varying amounts of the impurity in the different preparations. Thus, it is not necessary to invoke the hypothesis that the virus particles themselves vary in their physical properties from preparation to preparation.

Introduction

Among the animal viruses isolated thus far, rabbit papilloma virus is one of the few obtained in relatively pure form. In fact, the homogeneity of the virus preparations, as judged by several criteria, has been such as to justify favorable comparison of the virus with many so-called pure proteins.

The results of early studies by Beard and co-workers[2a,b] on twelve different preparations of purified virus, using the ultracentrifuge, the Tiselius electrophoresis apparatus, diffusion measurements, and the electron microscope, were interpreted by them as indicating homogeneity of the virus particles. All of the data fit into a unified picture indicating a uniformity of the virus particles with respect to size, shape, density and electrical properties.

In a later paper, Sharp, Taylor and Beard[3] reported on the density of the virus particles in solution. From sedimentation studies in solutions of varying density, they calculated that the virus particles contained about 58% water by volume. At the same time, they pointed out that the variations in sedimentation constant found earlier for different preparations of the virus were far beyond the experimental error. In a re-evaluation of the data, they attributed these variations in sedimentation constant in different preparations to natural differences in the virus particles, probably in their water content.

Inasmuch as the particles within each preparation appeared to have essentially the same physical properties and, furthermore, since each preparation was isolated from a pool of warts obtained at random from many rabbits, it seemed unlikely that the virus particles themselves had different physical properties in the various preparations. Therefore, an attempt was made to find an alternative explanation for the variations in sedimentation constant from preparation to preparation. Through the kindness of Dr. C. A. Knight of this Laboratory a limited amount of purified rabbit papilloma virus was made available. This communication presents the results of some physical chemical measurements made on this material and an alternative explanation for the discrepancies observed in the earlier sedimentation studies.

Materials and Methods

Several preparations of rabbit papilloma virus were used for these studies. All of the virus was obtained from warty growths of cottontail rabbits trapped in Kansas in different years. Details of the method of purification are given in a paper by Knight.[4] In general, the method is similar to that used by Beard and associates[5] and consists of a series of four or five cycles of alternate high- and low-speed centrifugation. M/15 phosphate buffer at *p*H 6.6 was used as a solvent during the isolation and for many of the physical chemical measurements. Ultracentrifuge studies were performed in both an air-driven ultracentrifuge of the Bauer–Pickels type and an electrically driven Spinco ultracentrifuge. Both ultracentrifuges were equipped with a Philpot–Svensson optical system. Electrophoresis experiments were conducted in a Perkin–Elmer Tiselius apparatus using a microcell and the Longsworth scanning optical system. An Ostwald type viscometer, specially designed for a low average shear gradient, was used for viscosity studies.

Ultracentrifuge and Viscosity Studies

All preparations examined in the ultracentrifuge showed a principal, sharp boundary with a sedimentation constant for the different samples between 269 and 290 *S*. In addition, most of the samples showed a faster and a slower component whose boundaries were much more diffuse than the main component. The faster component, which has a sedimentation constant of about 390 *S*, is probably similar to that observed in the early studies of Beard and Wyckoff.[6] The sedimentation constant of the slower component, whose presence has not been reported previously, is between 170 and 190 *S*.

Figure 1 shows the ultracentrifuge patterns and the sedimentation constants of the principal component of two different samples of rabbit papilloma virus. The amount of the slow moving component, sedimentation constant about 181 *S*, relative to the main one varies from one sample to another. Corresponding to the variation in the amount of the trailing component there is a variation in the sedimen-

(1) Presented before the Division of Biological Chemistry at the 116th National Meeting, American Chemical Society, Atlantic City, N. J., September 20, 1949.

(2) (a) H. Neurath, G. R. Cooper, D. G. Sharp, A. R. Taylor, D. Beard and J. W. Beard, *J. Biol. Chem.*, **140**, 293 (1941); (b) D. G. Sharp, A. R. Taylor, D. Beard and J. W. Beard, *Proc. Soc. Exp. Biol. Med.*, **50**, 205 (1942).

(3) D. G. Sharp, A. R. Taylor and J. W. Beard, *J. Biol. Chem.*, **163**, 289 (1946).

(4) C. A. Knight, *Proc. Soc. Exp. Biol. Med.*, **75**, 843 (1950).

(5) J. W. Beard, W. R. Bryan and R. W. G. Wyckoff, *J. Infect. Dis.*, **65**, 43 (1939).

(6) J. W. Beard and R. W. G. Wyckoff, *J. Biol. Chem.*, **123**, 461 (1938).

tation rate of the principal component. As shown in Fig. 1, the preparation having the smaller amount of trailing component has a decidedly higher sedimentation constant. The sample whose ultracentrifuge pattern is on the right in Fig. 1 was obtained from that shown on the left by lowering the *p*H to 3.5 by dialysis and then bringing it back to *p*H 6.6 followed by one cycle of alternate high- and low-speed centrifugation. In the region of the isoelectric point, about *p*H 5.0,[7] the virus is insoluble but it is readily soluble at *p*H 3.5. A considerable amount of the small component was removed by this lowering and raising of the *p*H, and there was a corresponding increase in the sedimentation constant of the main component.

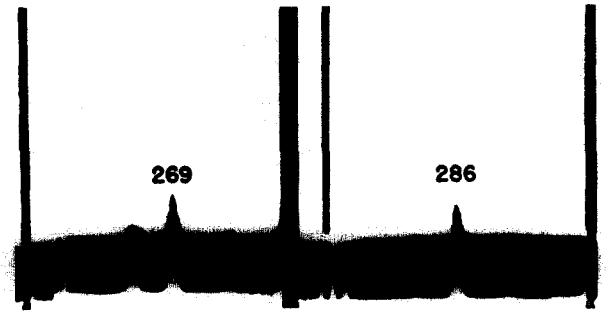


Fig. 1.—Ultracentrifuge patterns and sedimentation constants of two samples of rabbit papilloma virus in *M*/15 phosphate buffer at *p*H 6.6.

Another sample of partially purified virus with sedimentation constant of the main component equal to 284 *S* was subjected to two cycles of alternate high- and low-speed centrifugation in an attempt at further purification. The field used for centrifuging the virus was less than that used during the isolation in order to leave some of the trailing component in the supernatant. The results, presented in Table I, show that in solutions of essentially equal concentration the sedimentation constant of the principal component increased upon further centrifuging. Paralleling this increase in sedimentation constant there was a decrease in the reduced viscosity, η_{sp}/C, where η_{sp} is the specific viscosity and C is the concentration in g./cc.

TABLE I

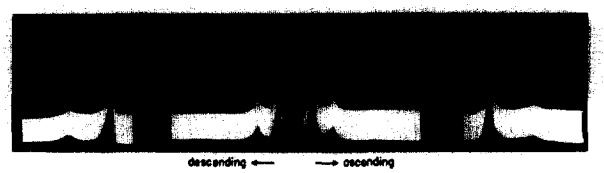
EFFECT OF CONTINUED CENTRIFUGATION OF PARTIALLY PURIFIED RABBIT PAPILLOMA VIRUS ON THE SEDIMENTATION CONSTANT AND REDUCED VISCOSITY

Sample	Concn., mg./cc.	*S*, svedbergs	η_{sp}/C, cc./g.
Original	2.1	284	17
1 Cycle	2.5	286	11
2 Cycle	2.3	290	11

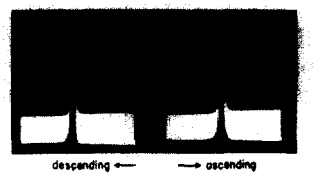
In agreement with the results of Sharp, Taylor and Beard,[3] it was found with one preparation that the sedimentation constant increased only slightly with dilution. Detailed studies could not be made because of the small amount of virus available. In some experiments the amount of virus available was so small that material could not be sacrificed for analysis.

Electrophoresis Studies

Two preparations of virus were studied in the Tiselius apparatus at *p*H 6.6 and the two boundaries observed are illustrated in Fig. 2. The sample whose electrophoretic pattern is shown in the upper part of Fig. 2 was recovered from the cell, dialyzed against veronal-acetate-chlorides buffer at *p*H 3.5, and once again studied in the Tiselius apparatus with the resultant single boundary shown in the lower part of Fig. 2. As indicated earlier, precipitation occurs in the vicinity of the isoelectric point and some material did not redissolve as the *p*H approached 3.5. However, there was still sufficient 180 *S* component left in solution at *p*H 3.5 to be visible in the ultracentrifuge which showed a pattern qualitatively not unlike the original. Further evidence of the presence of the small component was obtained by returning the virus solution by dialysis to *p*H 6.6. Again both components were observed in the ultracentrifuge and Tiselius apparatus although there was a definite decrease in the amount of the slow sedimenting component relative to the principal component. In the electrophoresis experiments mechanical compensation was not used, and the boundaries were moved from behind the glass plates by the electric field. It is not unlikely that the second component does not appear in the Tiselius pattern at *p*H 3.5 because its isoelectric point may be in that region and the boundary, consequently, did not migrate from behind the glass plates of the Tiselius cell.



Electrophoresis of rabbit papilloma virus in *M*/15 phosphate buffer at *p*H 6.6.



Electrophoresis of rabbit papilloma virus in veronal-acetate buffer, $\mu = 0.1$, at *p*H 3.5.

Fig. 2.

The results at *p*H 3.5 are very similar to those of Sharp, *et al.*,[7] and the mobility, 5.0×10^{-5} cm./sec./volt/cm., is consistent with the *p*H mobility curve obtained by these workers. As in the ultracentrifuge studies, a component not reported previously is seen in the Tiselius apparatus. The electrophoretic mobility of the main component at *p*H 6.6, about -2.3×10^{-5} cm./sec./volt/cm., observed in these experiments cannot be compared with that reported by Sharp and co-workers at *p*H 6.6 because of the different buffer system employed in the present studies.

Discussion

As Beard[8] and others have pointed out, the process of alternate high- and low-speed centrifugation is inefficient in separating particles of different sedimentation rates, and one preparation might differ from another in the amount of an impurity. If this impurity, though present in only small or barely detectable amount, were anisometric and more slowly sedimenting than the virus, its contribution to the viscosity of the solution might be sufficient to decrease the sedimentation rate of the virus. Furthermore, if different preparations of the virus contain the impurity in varying am3unts, then the solutions would have different reduced viscosities and the sedimentation rate of the virus would vary from preparation to preparation.

All of the samples of virus examined in the present study in both the ultracentrifuge and Tiselius apparatus show the presence of a more slowly sedimenting component in addition to the principal component, identified by Beard, *et al.*, as rabbit papilloma virus. In addition, the sedimentation studies indicate that the sedimentation rate of the virus component is dependent on the amount of the trailing component present in the preparation.

(7) D. G. Sharp, A. R. Taylor, D. Beard and J. W. Beard, *J. Biol. Chem.*, **142**, 193 (1942).

(8) J. W. Beard, *J. Immunol.*, **58**, 49 (1948).

According to the electron microscope studies of Sharp, *et al.*[2b] the virus particles are essentially spherical. Assuming the hydration of the virus is 58% by volume, a value of about 5.9 can be calculated for the intrinsic viscosity of virus solutions. In the present study the lowest value of the intrinsic viscosity is 14.5 and some values are as high as 21. Such high viscosities cannot be caused by spherical particles hydrated to the extent calculated by Sharp, *et al.* The viscosity studies suggest, then, that the virus solutions contain a viscous impurity. It is believed, therefore, that the variations in the sedimentation constants of the different samples examined in the present study are attributable to the presence of different amounts of a viscous impurity in the preparations rather than to inherent differences in the nature of the virus. This conclusion is based on the observation that the reduced viscosity of the solution decreases and the sedimentation constant of the main component increases as the amount of the trailing component decreases. In addition, the same virus preparation, on further purification, decreased in viscosity and the sedimentation constant of the principal component increased.

Lauffer and Stanley[9] in studies on influenza virus obtained results similar to those described here. The impurity in the preparations of influenza virus is believed to be a normal host component.[10] Information as to the nature of the impurity in the rabbit papilloma virus preparations is lacking at present because of the difficulty in obtaining sufficient material for study.

Despite the fact that the impurity is visible in both the ultracentrifuge and Tiselius apparatus, it is important to note that it is very inhomogeneous and that it may escape detection by these techniques if present in very small amounts. In that event viscosity measurements would be very useful in indicating the presence of the impurity. Neurath, *et al.*,[2a] obtained about 8.4 for the intrinsic viscosity of one of their preparations of virus indicating that that preparation contained less impurity than the preparations examined in the present study. However, the range of sedimentation constants reported by these workers is about the same as that observed in this study. Thus, it appears that most of the preparations of rabbit papilloma virus studied thus far contained virus particles of essentially uniform physical properties and variable amounts of an impurity rather than virus particles which varied in their physical properties from preparation to preparation.

(9) M. A. Lauffer and W. M. Stanley, *J. Exptl. Med.*, **80**, 521 (1944).

(10) C. A. Knight, *ibid.*, **80**, 83 (1944).

BERKELEY, CALIF. RECEIVED OCTOBER 6, 1950

NOTES

Salts of Substituted Piperidines and Pyrrolidines[1]

BY CARL T. BAHNER, MARVEL FIELDEN, LYDIA MOORE RIVES AND MADGE DEEL PICKENS

Since certain quaternary salts of pyridine and substituted pyridines have been found to damage sarcoma cells *in vivo*[2] it is of interest to determine whether the corresponding hydrogenated tertiary amine hydrohalide salts or the quaternary salts derived from N-alkylated piperidine produce similar effects. The study has been extended to include a few derivatives of pyrrolidine because of its close similarity to piperidine. Turner[3] and Lutz[4] and their associates, among others, have prepared several N-substituted piperidines, but little attention has been given to the quaternary salts.

The tertiary amine hydrohalide salts were prepared in some cases by reaction of equimolecular mixtures of the secondary amine and organic halide and in other cases by treatment of previously prepared tertiary amine with the proper acid. The quaternary salts were prepared in some cases by reaction of N-ethylpiperidine with the higher molecular weight organic halide and in other cases by treatment of a large tertiary amine with methyl or ethyl iodide.

(1) This investigation was supported in part by a research grant from the National Cancer Institute, of the National Institutes of Health, Public Health Service.

(2) Shear, *et al.*, in "Approaches to Cancer Chemotherapy," American Association for the Advancement of Science, F. R. Moulton, Editor, Washington, D. C., 1947, p. 236 ff.; *cf.* J. L. Hartwell and S. R. L. Kornberg, THIS JOURNAL, **68**, 1131 (1946).

(3) B. R. Carpenter and E. E. Turner, *J. Chem. Soc.*, 869 (1934); R. V. Henley and E. E. Turner, *ibid.*, 1182 (1931).

(4) Robert E. Lutz, *et al.*, *J. Org. Chem.*, **12**, 617 (1947).

Experimental

The methods of preparation are illustrated by the following examples. The melting points and analytical data on the products are listed in Table I.

1-(2-Phenylethyl)-piperidine Hydriodide.—A mixture of 11.6 g. of 2-phenylethyl iodide and 4.5 g. of piperidine evolved heat and set to a paste within one hour. Recrystallization from ethanol yielded 4.2 g. (26%) of white crystalline product.

1-Ethyl-1-(2-phenylethyl)-piperidinium Iodide.—A mixture of 2.5 g. of 1-(2-phenylethyl)-piperidine, prepared from the hydriodide by treatment with ammonium hydroxide and distillation of the oil at 145–150° at 10 mm., and 2.5 g. of ethyl iodide warmed slightly to initiate reaction, then allowed to stand 12 hours, yielded 2.5 g. (55%) of recrystallized white salt. Much lower yields were obtained when the reaction was attempted in a sealed tube at 100°.

Attempts to prepare 1,1-bis-(2-phenylethyl)-piperidinium iodide by heating of phenylethyl iodide with 1-(2-phenylethyl)-piperidine at 100° resulted in the formation of 1-(2-phenylethyl)-piperidine hydriodide instead of the expected product. Perhaps the quaternary salt was formed and broke down quickly by elimination of phenylethylene.

1-Ethyl-1-(*p*-chlorophenacyl)-piperidinium Bromide.—A solution of 4.6 g. of *p*-chlorophenacyl bromide and 2.6 g. of 1-ethylpiperidine remained clear after standing 3 weeks at room temperature, but addition of ethyl ether threw out