THE NATURE OF INTERGENERIC EPISOMAL INFECTION

By J. Marmur,* R. Rownd,† S. Falkow,‡ L. S. Baron,‡ C. Schildkraut,† and Paul Doty

DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY

Communicated May 24, 1961

Evidence has now been accumulated to bear out the hypothesis that interspecies genetic exchange will occur only among organisms which have similar deoxyribonucleic acid (DNA) base compositions. This has been found to be true among the T-even coliform bacteriophages,[1a,b] the *Brucellaceae*,[2] the *Bacillaceae*,[3] the *Neisseriaceae*,[4] and the *Enterobacteriaceae*,[5] the former three bacterial groups by means of transformation and the latter by means of transduction and conjugation. Recently, a different means of nonintegrated genetic exchange mediated by episomal infection[6, 7] has been shown to take place among the members of the *Enterobacteriaceae* which differ significantly in their DNA base compositions.[8a,b, 9]

The genetic determinants of bacteria normally comprise an integral part of the structure of the chromosome and are always present in one or another of their allelic forms. There is a class of genetic elements, however, which can be either present or absent and which, in the cell, can replicate synchronously or autonomously with respect to the bacterial genome. For these added elements, Jacob and Wollman have proposed the name *episomes*.[6]

The process of conjugation in the *Enterobacteriaceae* (which has been observed only among organisms of similar DNA base composition) involves a sexual differentiation which has been ascribed to the presence of a fertility factor in the donor strain (or male) and its absence in the recipient strain (or female). The fertility factor possesses remarkable properties in that it may exist either in an integrated state, in which it replicates as part of the bacterial genome (Hfr-male), or in an autonomous state, in which it behaves as if it were independent of the bacterial genome (F^+-male);[10, 11] hence, the fertility factor belongs to the episomic class of genetic elements. Although it appears that the integrated and nonintegrated states are mutually exclusive, unstable Hfr strains have been described in which both states appear to be in equilibrium[12] and in these variant strains the fertility factor is always attached at the same site of the bacterial chromosome when in the integrated state.[13] Two variants of this type have been isolated in which the fertility factor has incorporated a part of the bacterial chromosome one (F-Lac) which carries a determinant of β-galactosidase synthesis and the other (F-Pro) which carries a determinant of proline synthesis,[11] and is able to transfer these chromosomal segments to suitable F^- recipient strains. In this process of genetic transfer which has been termed F-duction, the fertility factor and the particular chromosomal segment are transferred exclusively and hence the genetic transfer is analogous to the process of transduction in which a temperate bacteriophage may transfer a part of the host genome to a recipient bacterium.[14, 15]

Escherichia coli and *Salmonella* (50% guanine + cytosine) will transfer several episomal elements to *Serratia marcescens* (58% guanine + cytosine).[8a, b] Since the buoyant density of DNA in CsCl has been found to be a function of its G-C content,[16, 17] the banding positions of the DNA of both parent strains will be different

in a CsCl density gradient; moreover, their band profiles indicate that they possess essentially no molecules of similar base composition in common. Thus the technique of density gradient centrifugation[18] provides a unique method of detecting genetic transfer between *E. coli* or *Salmonella* and *Serratia*. Furthermore, fractionation by preparative density gradient centrifugation of the DNA isolated from the episomally infected recipient makes possible the detection of relatively small amounts of DNA which would not be detectable in the unfractionated sample.

Genetic Evidence for Episomal Transfer.—A strain of *Salmonella typhosa* carrying the fertility factor, F, of *Escherichia* which harbors a segment of the bacterial chromosome governing lactose utilization (F-Lac) was used in this study. This organism is capable of transmitting donor ability and lactose utilization exclusively and with a relatively high efficiency to various species and strains of *Escherichia*, *Vibrio*, *Salmonella*, *Shigella*, and *Serratia*. The transfer of this episomal element has been found to be independent of any other genetic marker and the kinetics of transfer are similar to those found for the transfer of the sex factor in $F^+ \times F^-$ matings. It has been found that the episomal element of the recombinant culture replicates as a single unit independent of the host genome or at best undergoes a rapid alternation between the integrated and nonintegrated states.[7, 8a,b, 11] A more detailed description of this case of intergeneric episomal transfer has been reported in a separate communication.[8a,b]

The Detection of Donor DNA in the Recipient Organism.—The episomally infected *S. marcescens* strain was cultured from a single clone and the DNA isolated by the method of Marmur.[19] In addition, DNA was isolated from the *S. marcescens* parent strain and from an F^+ strain of *E. coli* by the same procedure. The DNA was banded in a CsCl density gradient in the Spinco Model E Analytical Ultracentrifuge at 44,770 rpm. When equilibrium was reached, photographs were taken using ultraviolet absorption optics. Figure 1 shows microdensitometer tracings of the resultant band profiles of the DNA isolated from the *S. marcescens* parent strain (A), the episomally infected *S. marcescens* (F-Lac) (B), and, to illustrate the correspondency in density of the new band appearing in (B) to that of the donor strain, a final tracing shows the band profile of the DNA isolated from an F^+ strain of *E. coli* (C). The band of heaviest density is that of a reference standard (N^{15}-labeled *Pseudomonas aeruginosa* DNA[20]) which is used to calculate the density of the other bands. It can clearly be seen that the unfractionated episomally infected *S. marcescens* DNA gives rise to two distinct bands in the CsCl density gradient, whereas the unfractionated *S. marcescens* parent strain does not. Furthermore, the buoyant density of the new band appearing in (B) corresponds to the buoyant density of the DNA isolated from the F^+ donor strain of *E. coli*. The buoyant density of the heavier band (1.718 gm/cm^3) corresponds to a G-C content of 58% (equivalent to that of the *S. marcescens* parent strain), whereas the lighter band (1.709 gm/cm^3) corresponds to a 50% G-C content (equivalent to that of the *E. coli* F^+ donor strain). The lighter band represents approximately 1% of the total DNA of the recombinant. When the DNA from the recombinant is thermally denatured and quickly cooled, the buoyant densities of both bands increase 0.015 gm/cm^3 in agreement with previous observations,[16] whereas the DNA isolated from the parent *S. marcescens* gave rise to a single band with a corresponding density increment under the same conditions.

The DNA isolated from the episomally infected *S. marcescens* was also studied after it had been fractionated using CsCl preparative density gradient centrifugation. This procedure was deemed advantageous in that it would provide a more sensitive method for the detection of DNA transferred from the donor strain to the recipient and it would also make possible a closer study of the transferred DNA by the removal of a large proportion of the normal DNA complement of the recipient *S. marcescens*. In a typical experiment approximately 300 μg of the DNA isolated from the recombinant was dissolved in a concentrated solution of CsCl and the density adjusted to approximately 1.710 gm/cm^3; this solution was then centrifuged in a lusteroid tube in the Spinco Model L Preparative Ultracentrifuge using the SW39 swinging bucket rotor. Normally the material was centrifuged at 35,000 rpm for approximately 24 hours and then the speed reduced to 20,000 rpm and the run continued at this speed for 4–5 days. At the end of this period the rotor was allowed to coast (unbraked) to a stop and the solution fractionated in an apparatus modeled after that of Szybalski[21] by puncturing a hole in the bottom of the lusteroid tube and collecting drops. After appropriate dilutions, absorbances at 260 mμ were measured on alternate fractions using a Beckman DU Spectrophotometer. The curve obtained by plotting absorbance versus fraction number is shown in Figure 2. Although no separate bands corresponding to the transferred DNA appeared in this plot, by centrifugation in the analytical ultracentrifuge of the indicated fractions (Fig. 2) selected from the less dense side of the band profile, the DNA introduced into the recipient *S. marcescens* could be readily resolved from its normal DNA complement. The results of this procedure are shown in Figure 3; in addition to confirming the presence of the band of buoyant density 1.709 gm/cm^3 as observed in the analytical ultracentrifuge on the unfractionated sample (Fig. 1B), the presence of a second band of buoyant

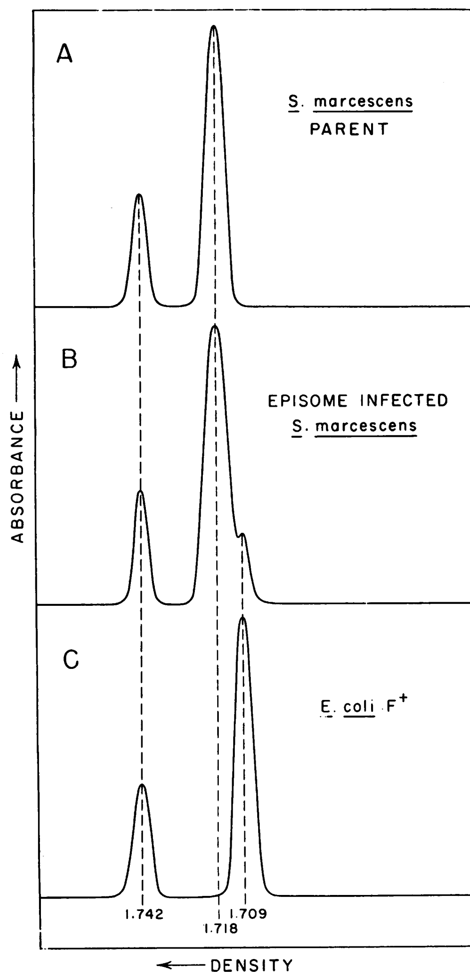

FIG. 1.—Band profiles of unfractionated DNA from parental and recombinant strains. Microdensitometer tracings of ultraviolet absorption photographs of samples at equilibrium in a CsCl density gradient at 44,770 rpm. The ordinate represents DNA concentration as a function of the distance from the axis of rotation. The band of buoyant density 1.742 gm/cm^3 (N^{15}-labelled *Pseudomonas aeruginosa* DNA) is used as a reference standard. See text for a description of samples.

density 1.703 gm/cm^{3} (44% G-C) was observed. This band is not detectable in the analytical ultracentrifuge run on the unfractionated DNA of the recombinant since it is present only to the extent of 0.1–0.2% of the total DNA sample.

In a control experiment the DNA isolated from the *S. marcescens* parent strain was fractionated and subsequently banded in the analytical centrifuge according to the above procedure. Although a band of buoyant density of approximately 1.709 gm/cm^{3} was observed in this case, it comprises less than 0.1% of the total DNA sample; similar bands have been observed in another preparation of *S. marcescens* DNA, as well as in several preparations of DNA from *Aerobacter aerogenes*.[22] In none of these preparations, however, was a band of buoyant density 1.703 gm/cm^{3} observed.

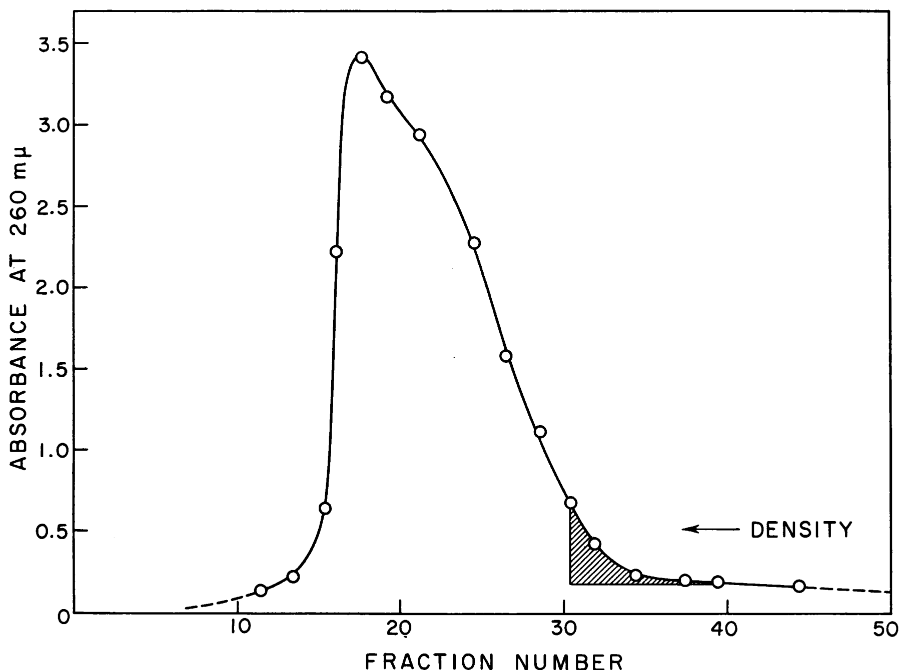

FIG. 2.—Preparative density gradient fractionation of DNA from *Serratia-coli* recombinant. Absorbances were measured at 260 mμ on a Beckman DU Spectrophotometer after appropriate dilution. Fractions were 0.06 ml each; above absorbances represent dilution of 1:2.67. Skewness of the band is an artifact resulting from the large amount of DNA which had to be banded in order to obtain sufficient amounts of the transferred DNA. For a description of the fractionation procedure, see text. Shaded area represents fractions subsequently centrifuged in analytical centrifuge (Fig. 3).

Discussion.—The nature of the vectors involved in the transfer of genetic information among bacterial species has been elucidated in the process of transformation,[23] transduction,[14] and conjugation.[24] In all of these processes of genetic recombination DNA is implicated as the underlying principle of genetic specificity of the transferred material. The process of episomal transfer has not been so extensively studied as a means of genetic exchange, particularly in regard to the nature of the episomal element. Genetic analysis has shown that the fertility factor is able to ensure the transfer of small chromosomal segments by a process

which is similar in many respects to that of specialized transduction. In addition, the fertility factor and the F-duced chromosomal segment are transferred exclusively and the episomal element may not be integrated into the genome of the recipient bacterium since the recombinants carry both parental alleles.[7, 11] In a recent communication,[25] Lavalle and Jacob have reported that several episomal elements are sensitive to the disintegration of P^{32} in the same manner as the genetic material of the chromosome. From the results of their study it is reasonable to conclude that episomal elements are high molecular weight DNA molecules.

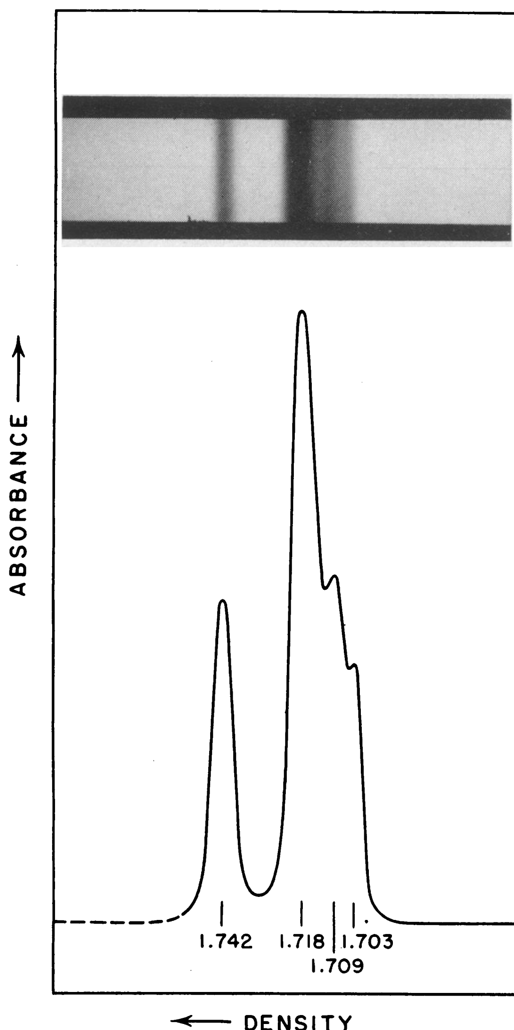

FIG. 3.—Band profile of fractionated DNA of *Serratia-coli* recombinant. Analytical ultracentrifuge run on fractionated *Serratia-coli* recombinant DNA. Fractions centrifuged are indicated in Fig. 2. Ultraviolet absorption photograph taken after equilibrium was reached at 44,770 rpm (top). Microdensitometer tracing of ultraviolet absorption photograph (bottom). The band of buoyant density 1.742 is used as a reference standard (N^{15}-labeled *Pseudomonas aeruginosa* DNA).

The evidence presented above clearly indicates that genetic exchange can occur among bacteria whose DNA base compositions are different. Furthermore, although cytoplasmic factors may be involved, it is clear that episomal transfer does involve a transfer of DNA from the donor to the recipient cell since separate bands corresponding in density to both parent strains are observed in the CsCl density gradient. Since the band width obtained in the CsCl density gradient is a function of the molecular weight of the banded DNA,[18] it is also evident that the transferred DNA is of a molecular weight comparable to that of the donor and recipient strains, since similar band profiles are obtained for the banded DNA of both parent strains and the transferred DNA. It is also reasonable to conclude that two types of high molecular weight DNA are transferred since two separate bands of different G-C contents are observed after the DNA isolated from the episomally infected recipient has been fractionated using CsCl preparative density gradient centrifugation. The results presented in this study are certainly consistent with the hypothesis that the genetic component of the episomal element is deoxyribonucleic acid.

No attempt will be made here to identify the two observed bands in terms of their genetic function; this is the object of a further study. It is striking, however, that the physical-chemical evidence is consistent with the genetic evidence in indicating the involvement of two distinct characters in the process of episomal transfer. In the absence of any positive identification of the functional nature of the two observed minor bands, any interpretation of this observation must remain purely a matter of speculation.

It is interesting that no bands of buoyant density intermediate between those of the donor and recipient parent strains are observed in the CsCl density gradient. If it is assumed that a genetic recombination between the episomal element and the *S. marcescens* host genome would result in a *hybrid* DNA of an average G-C content intermediate between those of the donor and recipient parent strains, then this *hybrid* should band between the two parent strains. Since no intermediate band was observed, even after the DNA isolated from the recombinant had been fractionated using CsCl preparative density gradient centrifugation, the evidence presented in this paper is consistent with the view that the episomal element is not integrated into the host genome by a process of genetic recombination, but rather replicates independently in the cytoplasm or in some unknown association with the host genome.

DNA isolated from bacterial sources shows a small degree of heterogeneity with respect to base composition and a few successful attempts have been made to fractionate nucleic acids with respect to differences in biological and physical-chemical properties. The interspecific transfer of genetic material among organisms which differ in base composition represents a unique method of *biological* fractionation. Since several chromosomal segments have already been F-duced and it seems likely that any gene of the bacterial chromosome may become incorporated into a fertility factor and, hence, exhibit the properties of episomal elements, intergeneric episomal transfer and subsequent fractionation in the CsCl density gradient may be used to isolate various regions of the donor genome.

The problem of contamination must be considered in a discussion of the results presented in this paper since the two bands observed after fractionation of the DNA isolated from the recombinant represent a relatively small fraction of its total DNA complement. For this reason the episomally infected *S. marcescens* was cultured from a single clone as described above. The buoyant densities of the episomal bands, as well as their relative proportions, were reproducible in DNA preparations from recombinants obtained from several different crosses. If contamination is to be assumed a factor in these results, then it must be postulated that a *double* contamination by two bacterial species whose respective G-C contents are 50 per cent and 44 per cent has occurred; moreover, this double contamination must occur in the same proportions. We feel that this is an improbable chain of events.

The possibility that other factors may be responsible for observed decrease in buoyant density of a small fraction of the DNA isolated from the recombinant must also be considered. For example, could protein-DNA aggregates account for the small fraction of DNA of lighter buoyant density observed in the CsCl density gradient? Extensive studies on the DNA isolated from *E. coli* K-12 under a wide range of conditions have always shown it to have the same buoyant

density in CsCl.[26] All unfractionated bacterial DNA preparations which have been banded in CsCl have been observed to have unimodal DNA distributions.[16,17,27,28] The DNA obtained from direct lysates of numerous bacterial preparations have always been observed to form unimodal, sharp bands of the same buoyant density as extensively deproteinized preparations.[29] Finally, all DNA preparations which have been examined to date, irrespective of the source of the DNA and the method used for its isolation, have been observed to fit the linear relationship established between buoyant density in CsCl and G-C content,[28] with the exception of the DNA isolated from the T-even coliform bacteriophages which contain an unusual base (5-hydroxymethylcytosine) which may be glucosylated to varying degrees.

Summary.—Genetic exchange can occur among organisms which differ in DNA base composition. The results presented in this study are consistent with the genetic evidence that episomal elements are deoxyribonucleic acid and that the transferred material is not integrated into the genome of the recipient bacterium. It has also been shown that the transfer of the (F-lac) episome involves two distinct species of DNA whose base compositions are 50 per cent and 44 per cent G-C respectively.

We should like to thank D. M. Green and C. Levinthal, as well as J. Lanyi, for valuable discussions and W. Torrey for his aid in performing some of these experiments.

* Department of Biochemistry, Brandeis University, Waltham, Mass.

† Predoctoral Fellow of the National Sciences Foundation.

‡ Division of Immunology, Walter Reed Army Institute of Research, Washington, D. C.

(*a*) Streisinger, G., *Virology*, **2**, 377 (1956). (*b*) Delbruck, M., and W. T. Bailey, Jr., in *Cold Spring Harbor Symposia on Quantitative Biology* (Cold Spring Harbor: The Biological Laboratories, 1946), vol. 11, p. 33.

[2] Schaeffer, P., in *Symposia of the Society for Experimental Biology* (London: Cambridge University Press, 1958), vol. 12, p. 60.

[3] Marmur, J., E. Seaman, and J. Levine (in preparation).

[4] Catlin, B. W., *Bacteriol. Proc.*, p. 90 (1961).

[5] Ravin, A. W., *Bacteriol. Rev.*, **24**, 201 (1960).

[6] Jacob, F., and E. L. Wollman, *C.R. Acad. Sci. (Paris)*, **247**, 154 (1958).

[7] Jacob, F., P. Schaeffer, and E. L. Wollman, in *The Tenth Symposium of the Society for General Microbiology* (Cambridge: The University Press, 1960), p. 67.

[8] (*a*) Carey, W., W. Spilman, and L. S. Baron, *Bacteriol. Proc.* (1960). (*b*) Falkow, S., J. Marmur, W. Carey, W. Spilman, and L. S. Baron, *Genetics* (in press).

[9] Nakaya, R., A. Nakahura, and Y. Murata, *Biochem. Biophys. Research Commun.*, **3**, 654 (1960).

[10] Lederberg, J., in *Abstracts of the Seventh International Congress on Microbiology* (Stockholm: Almqvist and Wiksell, 1958), p. 59.

[11] Jacob, F., and E. A. Adelberg, *C.R. Acad. Sci. (Paris)*, **249**, 189 (1959).

[12] Richter, A., *Genetics*, **42**, 391 (1957).

[13] Adelberg, E. A., and S. N. Burns, *Records Genetics Soc. Amer.*, **28**, 57 (1959).

[14] Zinder, N., in *Cold Spring Harbor Symposium on Quantitative Biology* (Cold Spring Harbor: The Biological Laboratories, 1953), vol. 18, p. 261.

[15] Morse, M. L., E. M. Lederberg, and J. Lederberg, *Genetics*, **41**, 142 (1956).

[16] Sueoka, N., J. Marmur, and P. Doty, *Nature*, **183**, 1429 (1959).

[17] Rolfe, R., and M. Meselson, these PROCEEDINGS, **45**, 1039 (1959).

[18] Meselson, M., F. W. Stahl, and J. Vinograd, these PROCEEDINGS, **43**, 1480 (1957).

[19] Marmur, J., *J. Molec. Biol.* **3**, 208 (1961).

[20] Marmur, J., and C. Schildkraut, *Nature*, **189**, 636 (1961).

[21] Szbalski, W., *Experientia*, **16**, 164 (1960).

[22] Rownd, R., J. Marmur, and P. Doty (unpublished results).
[23] Avery, O. T., C. M. MacLeod, and M. McCarty, *J. Exptl. Med.*, **79**, 137 (1944).
[24] Jacob, F., and E. L. Wollman, in *Symposia of the Society for Experimental Biology* (London: Cambridge University Press, 1958), vol. 12, p. 75.
[25] Lavalle, R., and F. Jacob, *C.R. Acad. Sci. (Paris)*, **252**, 1678 (1961).
[26] Schildkraut, C., R. Rownd, and P. Doty (unpublished results).
[27] Sueoka, N., *J. Molec. Biol.*, **3**, 31 (1961).
[28] Schildkraut, C., J. Marmur, and P. Doty (in preparation).
[29] Schildkraut, C., R. Rownd, J. Marmur, D. M. Green, and P. Doty (unpublished results).

---

*BIOPHYSICAL STUDIES OF BROAD BEAN MOTTLE VIRUS**

BY HIROSHI YAMAZAKI, JOHN BANCROFT, AND PAUL KAESBERG

DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN, AND DEPARTMENT OF BOTANY AND PLANT PATHOLOGY, PURDUE UNIVERSITY

Communicated by C. A. Elvehjem, May 19, 1961

Viruses are useful test objects for studying structural and functional relationships between proteins and nucleic acids. For these purposes it is desirable, at least for the present, to deal with viruses that are as small as possible with the expectation that small size and structural simplicity will go hand in hand. The members of the group of so-called *spherical viruses* perhaps approach this desideratum most closely. Among the most extensively studied are the plant viruses turnip yellow mosaic virus,[1] tomato bushy stunt virus,[2] wild cucumber mosaic virus,[3] southern bean mosaic virus,[4] and squash mosaic virus,[5] the bacterial virus ϕX174,[6] and polio virus.[7] All have molecular weights in the range 5–10 $\times$ 10^6 and nucleic acid content in the range 1.5–2.5 $\times$ 10^6 (in molecular weight units). Recently it has been shown that the molecular weight of bromegrass mosaic virus[8] is even lower —4.6 $\times$ 10^6—and that its content of nucleic acid is only 1 $\times$ 10^6.

The data to follow show that broad bean mottle virus (BBMV) is also quite low in molecular weight—5.2 $\times$ 10^6—and in nucleic acid content—1.1 $\times$ 10^6. This virus could become a useful complement to bromegrass mosaic virus since the two differ markedly in both amino acid and nucleotide composition. Indeed, because its yield from infected tissues is exceedingly high, broad bean mottle virus could be the more favorable subject for structural studies.

Broad bean mottle virus was first isolated and purified by Bawden, Chaudhuri, and Kassanis[9] who investigated some of its biological properties. Serological and some chemical properties have been studied by Wetter, Paul, Brandes, and Quantz.[10] Wittmann and Paul[11] determined its amino acid composition.

Methods.—Isolation and purification procedures: The virus was obtained from a stock culture kept by F. C. Bawden.[12] It was inoculated onto horse bean (*Vicia faba* L. var. broad windsor) and was isolated from infected leaves 3 weeks later.

Infected leaves, frozen at −25°C., were homogenized in a Waring blendor in cold potassium phosphate buffer (0.01 *M* in phosphate, pH 7). In some isolations as much as 0.01 *M* ascorbic acid was added to this solution in order to prevent oxidation of cellular constituents. An equal volume of a 1:1 n-butanol-chloroform mixture was added to the homogenate and the resulting mixture was shaken gently for 15 min. The emulsion was broken by a low speed centrifugation. The aqueous layer was removed and was immediately centrifuged in a No. 30 rotor in a Spinco Model L preparative centrifuge at 15,000 rpm for 15 min. The supernatant was then centrifuged at