examinations. For this reason studies of the occurrence of lymphocytes with bilobed nuclei appear to be an unusually promising means of identifying potentially harmful operations *before* gross overexposure can occur.

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Virus Strains of Identical Phenotype but Different Genotype

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Delbruck and Bailey (*1*) noticed an anomaly in the lysate of bacteria which was obtained by mixedly infecting the B strain of coli with the bacterial viruses T2 and T4. Subsequently, Luria (*2*) found this anomaly to be even more pronounced when he repeated Delbruck's experiment—using, however, virus T2 that had been exposed to ultraviolet irradiation.

When we undertook experiments in an attempt to understand this anomaly, we were led to the following result: If we infect a culture of the B strain of coli mixedly with the bacterial viruses T2 and T4 and incubate to permit lysis of the bacteria, there are present in the lysate 3 easily distinguishable types of bacterial viruses. Two of these, as expected, behave like the original parent strains T2 and T4, i.e., one of them behaves like T2 inasmuch as it is unable to attack the mutant strain B/2 (which is resistant to T2) but is able to grow in the mutant strain B/4 (which is sensitive to T2); the other behaves like T4, being unable to attack B/4 (which is resistant to T4) but is able to grow in B/2 (which is sensitive to T4). The third type of virus present is phenotypically like T4 inasmuch as it is capable of multiplying in the strain B/2 (which is sensitive to T4), but it is genotypically like T2 inasmuch as, after one passage in the strain B/2, it is no longer capable of growing in it but is capable of growing in the strain B/4 (which is sensitive to T2).

The presence of this third type of virus, which may be called "latent T2," can be demonstrated in the following manner: We add to a culture of the B strain of coli viruses T2 and T4 in ratios corresponding to 10 T2 and 10 T4 virus particles per bacterium, incubate to permit lysis of the bacteria, and then filter the lysate.

If we plate a sample of this lysate on agar that is inoculated with the strain B/4 (which is sensitive to T2 but resistant to T4), those virus particles contained in the lysate which have the phenotype T2 will show up as plaques on these plates. T4 virus particles will not give plaques on this plate because B/4 is resistant to T4. The number of plaques is thus a measure of the number of T2 particles in the lysate.

Using a sample of the lysate, we determine in this manner the number of plaques obtained on an agar plate inoculated with the strain B/4. When we repeat this experiment—with the difference that before plating on the B/4 plate we add to the sample of our lysate a certain quantity of the strain B/2, allow 5 min for absorption, dilute with broth, and incubate for 1 hr to permit lysis of the bacteria—then we obtain a ten to twenty-five times larger number of plaques on the B/4 plate.

This phenomenon appears to show that there is present in our lysate a virus (the "latent T2") which is capable of multiplying in B/2 and subsequently forming plaques on B/4. In order to account for our observation, the concentration of the "latent T2" in the lysate would have to be about 10% of the concentration of T2. We were not able to obtain, after one passage in B/2, any appreciable further growth in B/2 of our hypothetical "latent T2." Before drawing the conclusion that the presence of a "latent T2" is in fact responsible for our phenomenon, it is necessary to exclude alternative explanations.

As an alternative explanation of our observation, it appeared a priori conceivable that our lysate contains aggregates of virus particles formed by a T2 and a T4 particle that stick together. Such aggregates might then perhaps be capable of entering into a bacterium of the strain B/2 (by virtue of their T4 component) and, once inside, both virus particles T2 and T4 might then be able to multiply, and thus to produce the observed phenomenon. We were able to rule out this possibility, however, by performing the following experiment.

We add to a sample of our lysate a certain quantity of B/2, using an excess of B/2 so that independent infection of one bacterium by more than one virus particle can be neglected. We then allow 5 min for absorption and plate on an agar plate that has been inoculated with both B/2 and B/4. If there are present any B/2 bacteria into which has entered an aggregate of virus particles composed of T2 and T4, and in which both viruses will grow, then a certain number of clear plaques centering around such bacteria (which yield both T2 and T4) should develop on the agar plate. We were not able to find any such clear plaques, however, and found only turbid plaques (in which either the B/2 is lysed by T4 or the B/4 is lysed by T2). This rules out the alternative explanation of our phenomenon.

We ascertained that our phenomenon is produced under conditions in which we use an excess of B/2, so that independent infection of one bacterium by more than one virus particle can be neglected. We also ascertained that our phenomenon is not produced if, in place of our lysate, we use a mixture of T2 and T4.

We are thus led to conclude that the phenomenon described is due to virus particles that have the phe-

notype of T4, but the genotype of T2. The properties of this "latent T2" virus would seem to merit investigation.

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Paper Disk Columns in Glass Chromatographic Tubes

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The use of filter paper in selective adsorption experiments, probably first described by Schönbein (*1*), has become a powerful tool in research, especially since the studies of Consden, Gordon, and Martin (*2*). Although in most modifications of paper chromatography only very small amounts of material can be resolved, Mitchell and his colleagues must be credited with preparative applications; recently these authors carried out successful enzyme separations in their chromatopile (*3, 4*).

Up to the present time no use has been made of the classical Tswett glass tube packed with filter paper disks, for the following reason. Although a homogeneous, satisfactory column may readily be prepared from a powder, on the contrary, channeling and gross irregularities in the flow of solutions become manifest when paper disks are stamped into a cylindrical tube.

It was found that this difficulty can be overcome if the size of the disks is adapted to the average diameter of a good cylindrical tube by cutting the paper with unusually high precision. For this purpose a stainless steel die and a corresponding punch must be available by means of which a supply of precision-cut paper can be obtained. About 15 paper disks were cut simultaneously with our die when Eaton and Dikeman No. 615 filter paper was used. The same cutting device can, of course, be used to prepare paper for several glass tubes,[2] represented in Fig. 1, which possess similar diameters.

For example, the diameters of 2 tubes (measured in different directions and at various levels inside the tube) varied, respectively, between 1.716–1.730 in. (4.359–4.394 cm) and 1.730–1.736 in. (4.394–4.409 cm). The diameter of the die was 1.73064 in. (4.396 cm), and that of the punch was 1.7299 in. (4.3938 cm). If the average diameter of the glass tube was, for example, 1.68 in. (4.27 cm), the even flow of the solvent was markedly distorted.

In order to pack the chromatographic tube, the procedure shown in Fig. 2, upper part, is recommended. Each portion (20 paper disks, for example) is somewhat loosened up by ruffling the edges with the thumb, inserted a few centimeters deep into the horizontal glass tube and pushed in further with a smooth, cylindrical pestle (diameter, about 1 mm smaller than that of the tube), while the other hand is holding firm both parts of the glass tube (*cf.* Fig. 1) and exerting pressure. When the paper has reached its proper location, considerable pressure is exerted with the pestle for a moment while it is rotated by about a quarter turn. A column 20–25 cm high can be built up in about 15 min.

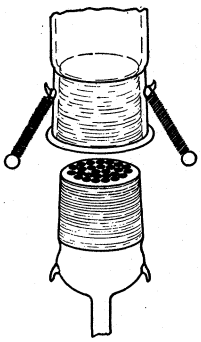

FIG. 1.

When suction of an ordinary water aspirator is applied, either aqueous or nonaqueous solutions flow evenly through a paper column which has been packed in the described way. The duration of the chromatographic experiment will then not exceed that required for a powder column of similar dimensions.

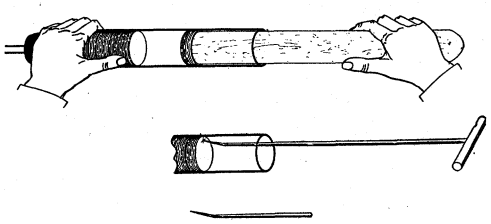

FIG. 2. Packing and removing a filter disk column. Left hand holds the ground glass section (*cf.* Fig. 1); dark area on left indicates position of the rubber stopper by means of which the tube will be attached to a suction flask.

Because of the tight contact between glass and paper, only short and broad columns can be extruded with a pestle or removed by means of a long-stem corkscrew; in general, however, the procedure as indicated in Fig. 2 (lower part) is recommended. About 1-cm sections of the column are rapidly taken out by using a sharply pointed and slightly bent steel pick which has a tempered end about 5 cm long.

The proposed technique shares the advantages of ordinary powder column experiments—i.e. those of a reasonably closed system—but has the disadvantage that the basic equipment requires the services of a precision mechanical shop.[3]

[1] Contribution No. 1436.

[2] The model used is the same as proposed earlier (*5*) and is manufactured by the Scientific Glass Apparatus Co., Bloomfield, N. J.

[3] We are indebted to J. H. Sturdivant for the facilities of the workshops under his direction.