grown in fertilized Wongan loamy sand (*p*H 5.0 in 1:5 0.01*M* $CaCl_2$, clay content 13 percent and a water content at field capacity of 9 percent) for approximately 2 weeks. Calcium-45 (3 μc per gram of soil) was mixed thoroughly through the soil before germination. The plants were grown under controlled conditions of temperature, light, and humidity; containers were made up to weight with water once, then twice daily. Autoradiographs were obtained every 2nd day, and the calcium content on acid-digested plant material grown as a control under identical conditions was determined by atomic absorption spectrometry (*5*). The soil solution was extracted by centrifugation (*6*).

Beginning on the 5th and definitely obvious by the 7th day, slightly lighter areas on either side of the root developed on the autoradiographs (Figs. 1a). These areas continued to widen until the 14th day, at the end of the experiment. The well-delineated boundary of the depletion zone which has been reported for molybdenum (*7*), phosphorus (*8*), and zinc (*4*) was not apparent, which suggests that the concentration gradient between the soil and the root was appreciably affected by mass flow.

The observation of a depleted zone around the root seems anomalous in view of the calculated possible mass flow contribution of more than ten times the plant absorption, which should cause calcium to accumulate at the root surface. Further conflicting evidence came from the fact that transpiration rates had little effect on calcium absorption in a duplicate experiment: plants transpiring at high rates had calcium contents (270 μg per plant) similar to those transpiring at one-third of the high rates (245 μg per plant), while maintaining similar growth.

The first anomaly may be related to the fact that the depletion zone around the root shown on the autoradiographs is a measure of the total ^{45}Ca. The absence of activity near the root indicates that ^{45}Ca has been reduced to a low level and, for this to happen, considerable quantities of exchangeable calcium must have been removed from the colloids within the depletion zone. When such quantities of ^{45}Ca are displaced from the soil surfaces in the vicinity of the root, the calcium concentration in the soil solution should initially increase, but will subsequently be modified by plant uptake and back diffusion. The quantity of calcium appearing in the plant is considerably less than a conservative estimate of that removed from the depletion zone, so that calcium must be moved away from the root by back diffusion or the downward movement of water following irrigation. However, ^{45}Ca in solution may be at a greater concentration near the root than in the bulk soil solution and still not be detected in the autoradiographs because the total ^{45}Ca concentration in the region has been appreciably decreased.

The small effect of transpiration may be associated with the fact that the accumulation of calcium at the root surfaces cannot be calculated simply by assuming that the soil solution concentration is constant in the larger pores carrying water to the root. If the soil pores were regarded as a simple capillary system, the ions in the soil solution would be moved toward the plant root, and when more water was added to the plant system, ions would not be available to desorb calcium from charged sites on the colloid to replenish the soil solution. Thus, particularly in the larger pores, water with a much lower ^{45}Ca content may be carried to the root. Increasing transpiration therefore may not appreciably increase the movement of ^{45}Ca to the root.

Irrespective of the detailed method of estimating the mass flow contribution, the fact remains that water flowing to the plant root will be carrying some calcium into the surface region, and yet the overall effect is that mobilized exchangeable calcium has moved away from the root. The situation at the boundary of the depletion zone is very complex, and the processes of mass flow and back diffusion may each occur concomitantly, but their relative effects will vary in relation to the pore size. It would be expected that the coarser pores with a low ratio of surface to volume would be mainly involved in mass flow and finer pores with considerably larger ratios of surface to volume may be the principal path for back diffusion.

Despite the problems in calculating the mass flow contribution to calcium movement, the most significant feature of the autoradiographs presented is that a large amount of the exchangeable calcium in the region adjacent to the root has been mobilized in 5 days, and it appears likely that hydrogen ions or possibly other substances from the root are implicated. This observation indicates how profoundly the plant root alters its immediate environment and suggests that much of the work seeking to analyze movement of ions to root surfaces needs reappraisal.

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Nucleic Acid Molecules: New Microdiffusion Technique for Visualization

Abstract. *A microdiffusion technique, developed for visualization of nucleic acid molecules in the electron microscope, requires less than 0.01 microgram of nucleic acid. Although originally developed for free nucleic acids, the method can be applied to virion suspensions for direct visualization of their genomes; less than 10^{10} virions per milliliter are required. Results agree well with those yielded by the diffusion technique of Lang, Kleinschmidt, and Zahn.*

The length and molecular weight of intact viral nucleic acid molecules have been successfully measured by the technique of Kleinschmidt and Zahn (*1*), in which macromolecules are adsorbed and fixed in a structureless protein monolayer before transfer to the grid of an electron microscope for visualization. In the original procedure (*1*), DNA molecules were spread on water; subsequently (*2*) the molecules were allowed to attach to the monofilm by diffusion-

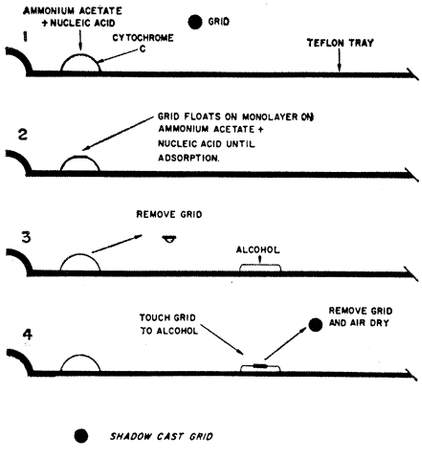

Fig. 1. Essential procedure of the new technique.

controlled adsorption, and shearing forces during spreading and convection effects were thus virtually eliminated.

Unfortunately, both techniques require relatively large quantities of purified nucleic acid, although the diffusion technique (*2*) requires concentrations 100 times lower than those considered optimal for spreading (*1*). However, at levels of about 2×10^{-8} g/ml, the lower concentration still amounts to 10 to 100 μg of purified material, depending on the dimensions of the specimen trough used for diffusion. These concentrations are reasonable for preparations of bacteriophages and some plant viruses, but they represent approximately 10^{12} or more particles of animal viruses for the small RNA- and DNA-containing agents if one assumes that each particle will yield its full complement of 2 to 3×10^6 daltons of nucleic acid. For many viruses, starting materials must comprise at least 1 liter of high-titered tissue-culture fluid.

We have developed a microdiffusion technique that requires only 0.01 μg of nucleic acid. Small droplets of the nucleic acid in a solution of 0.2*M* ammonium acetate are delivered by pipette to a clean sheet of Teflon; we use an inexpensive sheet measuring 37.5 by 26.2 cm, with a 1.2-cm side, but many shallow containers are suitable. The Teflon surface inhibits spreading of the solution, and a nearly spherical droplet is obtained.

The essential procedure is shown in Fig. 1. A needle dipped briefly into a solution of cytochrome c (powder: water, 1 : 1) is touched to the surface of the droplet; a monofilm is formed toward which the nucleic acid molecules diffuse. After 20 or 30 minutes the film is picked up directly on a carbon-coated electron-microscope grid. The liquid adhering to the grid is touched to the surface of absolute alcohol for removal of water, and the preparation is then shadow-cast with a thin layer of platinum-palladium evaporated at a low angle from two directions normal to each other. Each droplet provides the material for a single observation, but many experiments can be carried out in droplets placed side by side on the sheet. The optimum amount of cytochrome c to be conveyed by needle to the surface should be determined by preliminary experiment.

A density-gradient band containing less than 10^{10} particles per milliliter, of viruses such as SV40 or the adeno-associated satellite, is often more than sufficient nucleic acid (Fig. 2b); this is true also if the physical properties of the virions being studied are such that their genomes can be released readily by treatment with a high concentration of salt such as 12*M* ammonium acetate (Fig. 2, a, c, and d). With droplet volumes as small as 0.05 ml, electron-microscope examination of nucleic acid from density-gradient fractions can be correlated with determinations of density, infectivity, and other biological properties without squandering of valuable reagents. When virions are recalcitrant to treatment with high concentrations of salt, their genomes may be released sometimes by other procedures such as treatment with isoamyl acetate (*3*).

We have applied our technique to papovaviruses (SV40, rabbit papilloma, human papilloma), to adeno-associated satellite virus, to purified DNA obtained from SV40 and adenovirus, and to RNA from bacteriophage R17. The results have been encouraging and compare well with those yielded by established techniques (*1*, *2*).

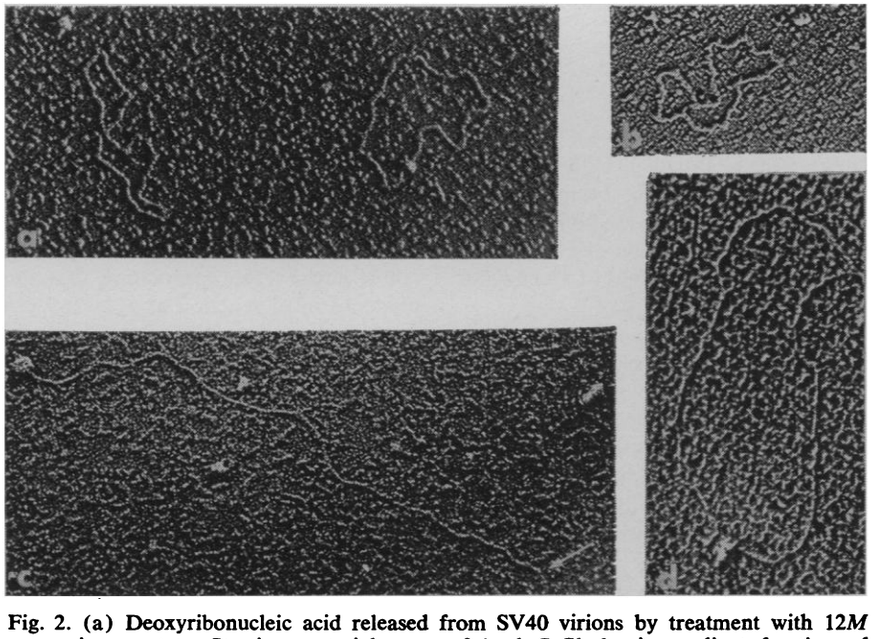

Fig. 2. (a) Deoxyribonucleic acid released from SV40 virions by treatment with 12*M* ammonium acetate. Starting material was a 0.1-ml CsCl density-gradient fraction of purified virions with a particle count of 10^{10} per milliliter. Cyclic monomers represent complete genomes; contour length, 1.6 μ (× 44,000). (b) Cyclic DNA extracted from purified SV40 virions; microdroplet contained 0.007 μg of DNA (× 44,000). (c) Linear monomer DNA released from rabbit papilloma virus by treatment with 12*M* ammonium acetate. Molecule represents complete genome; 2.4 μ long (× 44,000). (d) Cyclic monomer DNA released from human wart virions by treatment with 12*M* ammonium acetate. Starting material was a 0.1-ml CsCl density-gradient fraction of purified virions with a particle count of 10^{10} per milliliter. Molecule represents complete genome; contour length, 2.6 μ (× 50,000).

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