

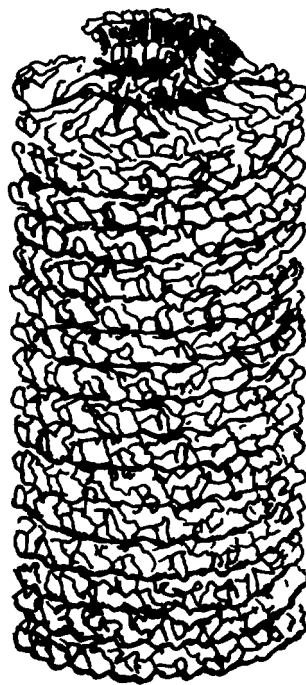
FERROFLUIDS

a new alignment technique

Typical neutron-scattering experiments on particles in solution yield only one-dimensional (spherically averaged) data because the scattering particles are randomly oriented. Such data can provide a model for the general shape and the boundaries of the scattering particles, but often more than one model is consistent with the data. However, if the particles are partially ordered by being given a definite orientation, the resulting scattering data contain more detailed structural information.

Alignment in one dimension has been achieved for the rod-shaped tobacco mosaic virus by applying shearing forces to an aqueous gel of the viruses. Two-dimensional x-ray-diffraction data for the aligned viruses led to a complete three-dimensional structure at a resolution of 3.6 angstroms (Fig. 1). Although that result is very impressive, the technique is not applicable to many biological structures.

Here we present preliminary studies of a new technique for aligning elongated biological assemblies in solution. The technique involves dispersing the assemblies in a ferrofluid (a fluid in which magnetic particles are suspended) and applying a moderate magnetic field. (Note that because the magnetic particles generally contain iron, which absorbs x rays strongly, the ferrofluid alignment technique is applicable to neutron-scattering experiments but not to x-ray-scattering experiments.) Magnetic forces cause the moments, or spins, of the magnetic particles to align along the direction of the mag-



STRUCTURE OF TMV

Fig. 1. The rod-shaped tobacco mosaic virus is about 3000 angstroms long and 180 angstroms in diameter. Its structure at a resolution of 3.6 angstroms was deduced from x-ray diffraction data for an aqueous gel of viruses. The viruses were oriented in one direction by shearing forces. The viral genetic material (RNA) is enclosed within a protein coat consisting of a helical array of many copies of a single protein species. The distance between the turns of the helix is 23 angstroms.

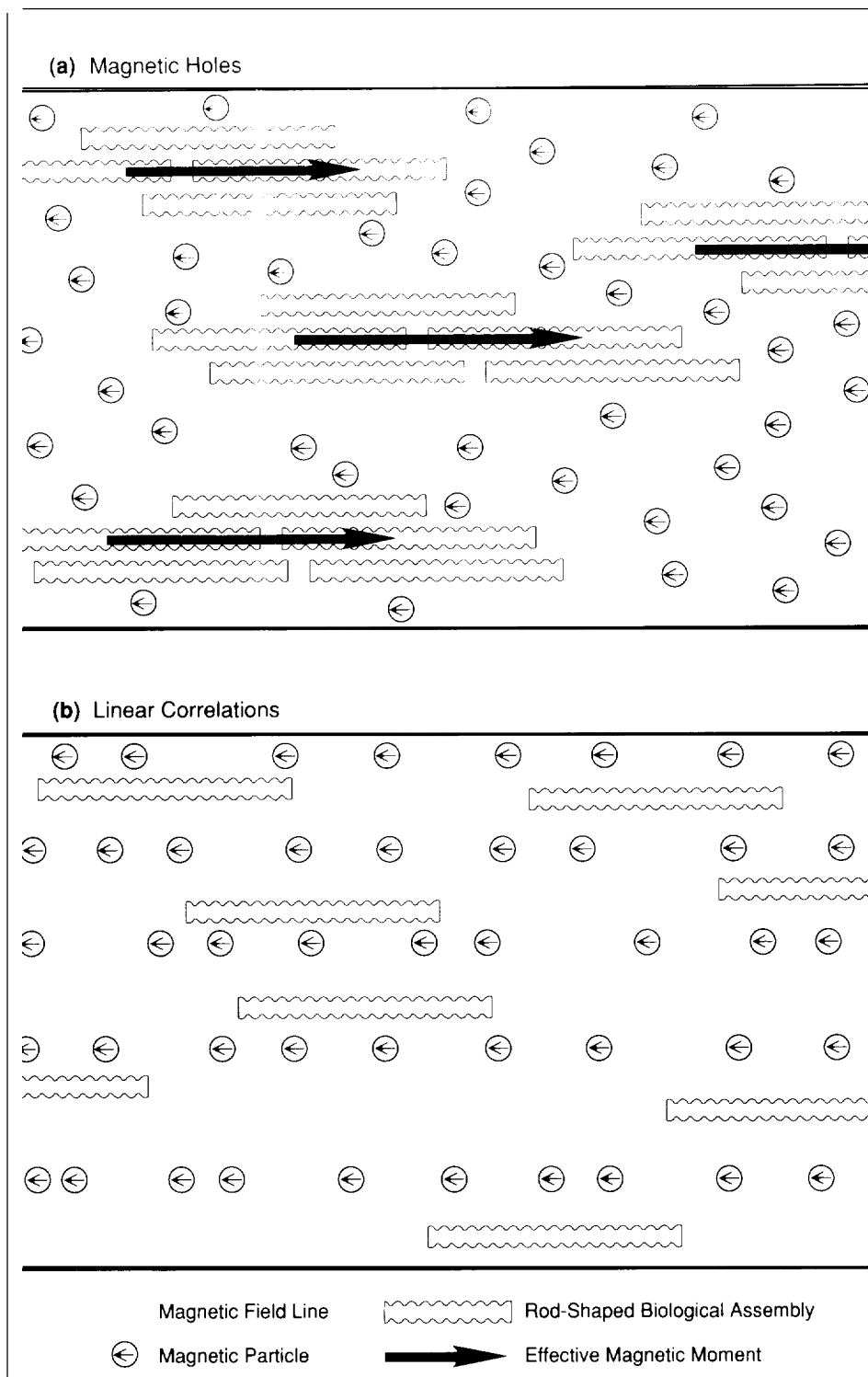
netic field, and their alignment, in turn, causes alignment of the elongated biological assemblies. Some biological assemblies have intrinsic diamagnetic moments and will therefore align along a magnetic field in the absence of mag-

netic particles. However, very strong magnetic fields are usually required. In contrast, the ferrofluid technique requires no intrinsic magnetic properties of the biological assemblies and only moderate magnetic fields.

We have tested the ferrofluid technique on two viruses, the tobacco mosaic virus (TMV) and the tobacco rattle virus (TRV), and have obtained neutron-diffraction data that testify to its success. Our tests focused not only on obtaining a high degree of alignment but also on understanding how the alignment comes about. Figure 2 shows two possible mechanisms. One possibility is that the biological assemblies act like "magnetic holes." That is, by displacing the ferrofluid, they acquire effective magnetic moments equal in magnitude and opposite in sign to the sum of the moments of the magnetic particles in the displaced ferrofluid. The effective moments then align along the applied magnetic field. In our experiments, however, the effective moments would be large enough to cause alignment of the assemblies in moderate magnetic fields only if the assemblies existed as ordered domains, or aggregates. Then each domain becomes a magnetic hole oriented along the applied field (Fig. 2a). The other possible mechanism of alignment invokes linear correlations among the magnetic particles, that is, a tendency for them to line up in rows along the applied field. Because disrupting those linear correlations would require energy, the non-magnetic elongated assemblies also tend to line up, with their long axes along the field, in rows between the rows of magnetic particles (Fig. 2b). It seems likely at this time that both alignment mechanisms operate to different degrees depending on the relative concentrations of magnetic particles and biological assemblies and whether the solution conditions favor the formation of ordered domains of the biological assemblies.

POSSIBLE MECHANISMS OF ALIGNMENT IN A FERROFLUID

Fig. 2. Elongated biological assemblies dispersed in a ferrofluid align along a moderate applied magnetic field even if they lack intrinsic magnetic properties. Shown here are two possible mechanisms of alignment. (a) Ordered domains of the biological assemblies act like magnetic holes. In other words, the domains acquire effective magnetic moments equal in magnitude and opposite in direction to the sum of the magnetic moments of the magnetic particles they displace. The effective magnetic moments cause the biological assemblies to align along the field. (b) Long-range magnetic correlations among the magnetic particles cause them to align in rows along the applied field. Lacking the energy to disrupt the linear correlations, the elongated biological assemblies also align along the field.

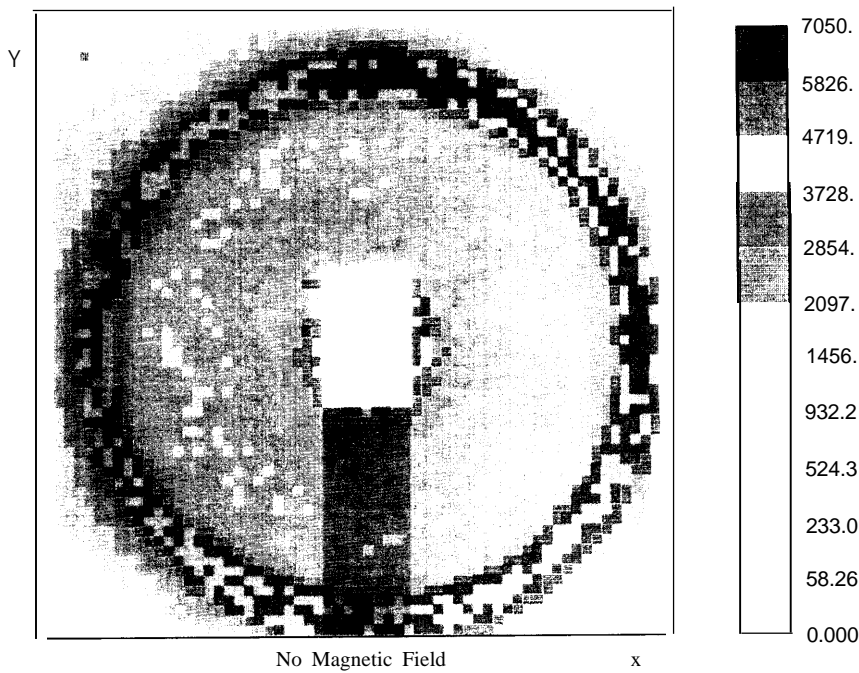


The magnetic particles in the ferrofluid would normally contribute to the observed scattering intensity. However, that contribution can be eliminated by matching the scattering-length density of the magnetic particles to that of the solvent. By a happy coincidence of nature, the neutron scattering-length density of the ferromagnetic material magnetite is quite close to that of D_2O . As is well known, particles of magnetite align with an applied magnetic field. To achieve a

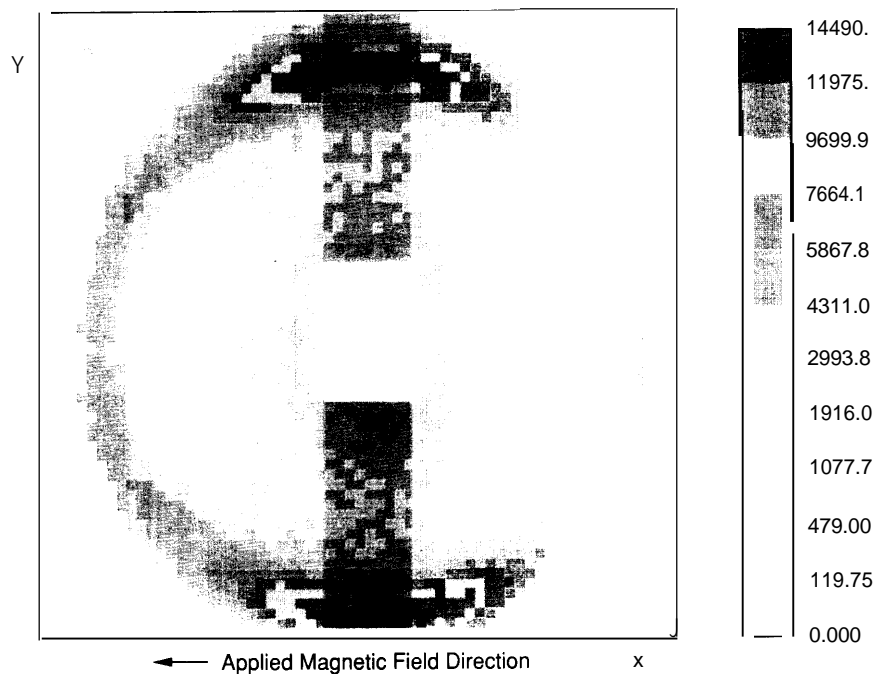
reasonably homogeneous suspension of magnetite particles in D_2O , the particles must be coated with a detergent surfactant. The detergent must be carefully chosen, however, to avoid its degrading the biological assemblies. Through extensive electron microscopy we identified a detergent that did not degrade TMV or TRV. We then deuterated the detergent to match the scattering-length densities of magnetite and D_2O .

To find out how well our ferrofluid

(a) Isotropic Diffraction Peak at 363 angstroms



(b) Anisotropic Diffraction Peak at 363 angstroms



SMALL-ANGLE NEUTRON-SCATTERING DATA FOR TMV IN A FERROFLUID

Fig. 3. Color-coded intensity data for neutrons scattered from tobacco mosaic virus dispersed in a ferrofluid. The intensity corresponding to the color of a detector pixel is related to the number of neutrons detected in the pixel. (a) In the absence of a magnetic field, the scattering is isotropic and a diffraction peak (a circle of high intensity) appears at a scattering angle corresponding to a distance of 363 angstroms. The diffraction peak reflects the packing distance between individual viruses in ordered domains. (b) When a magnetic field of 0.3 tesla is applied along the x direction, the diffraction peak appears only in the y direction, that is, perpendicular to the applied field. That result means that the packing distances are aligned perpendicular to the field and the long axes of the viruses are aligned along the field. The degree of alignment cannot be explained by intrinsic diamagnetism of the viruses and must therefore be caused by the ferrofluid.

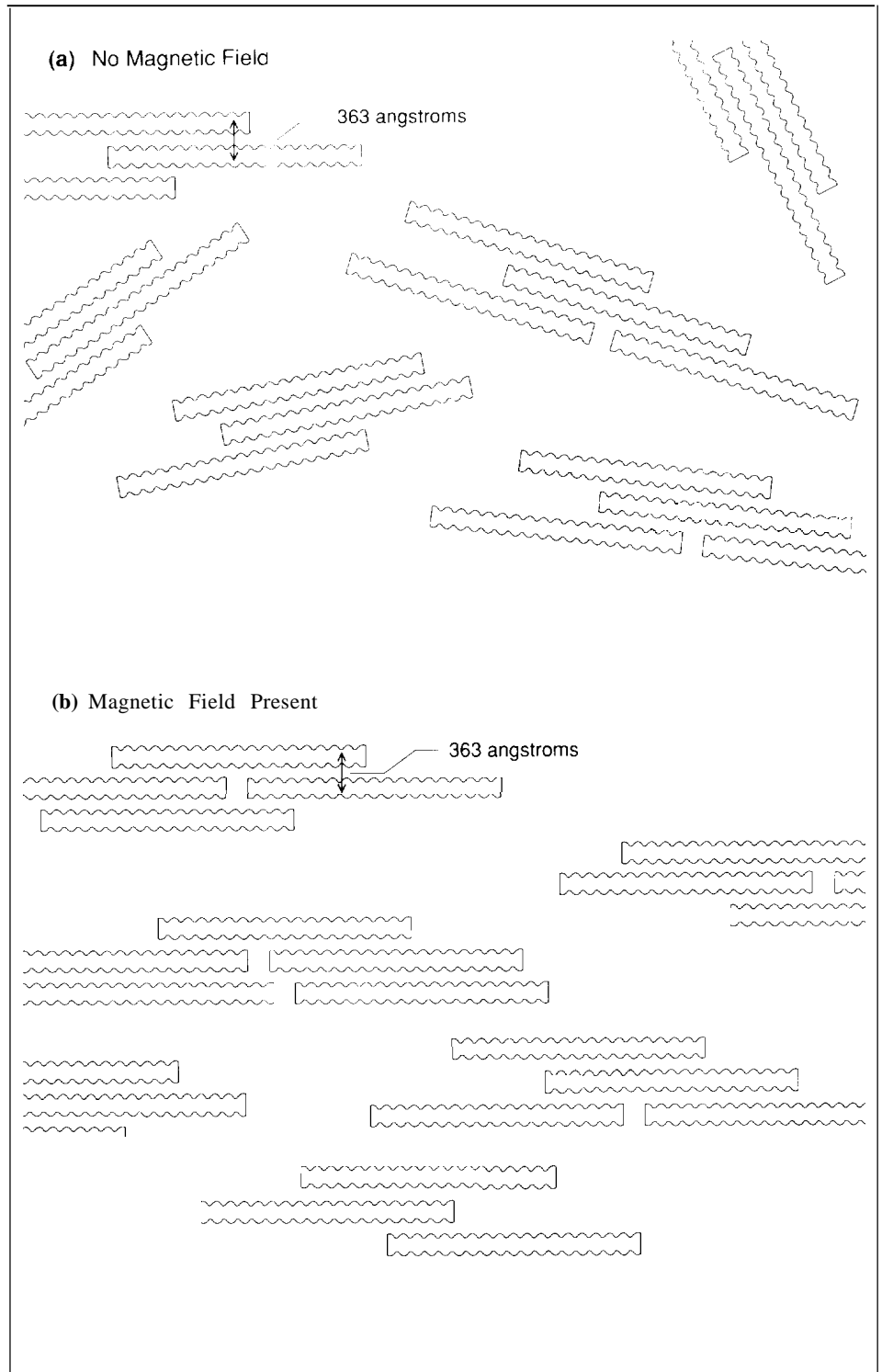
worked, we obtained neutron-scattering data for 0.06-volume-fraction dispersions of each virus in the ferrofluid. In the absence of a magnetic field, the scattering from TMV was isotropic, as expected (Fig. 3a). We also saw a diffraction peak corresponding to a distance of 363 angstroms. That diffraction peak arises because the viruses formed ordered domains in which the packing distance was 363 angstroms (Fig. 4a). (The packing distance is determined by

a repulsive electrostatic force between the viruses and varies with the ionic strength of the solution.) In a modest magnetic field of 0.3 tesla, the scattering was anisotropic, and the diffraction peak at 363 angstroms appeared only in the direction perpendicular to the field (Fig. 3b). Those results suggest that the TMV domains were aligned in such a way that the long axes of the viruses were parallel to the applied field.

If the TMV domains do so align,

ORDERED AGGREGATES OF TMV IN A FERROFLUID

Fig. 4. Tobacco mosaic viruses tend to form ordered domains in solution, as shown in the electron micrograph below. The viruses within a domain are kept at a certain distance by electrostatic forces. That distance depends on the pH and ionic strength of the solution and in our experiments was 363 angstroms. (a) In the absence of a magnetic field, the domains are randomly oriented in a ferrofluid. (b) An applied magnetic field aligns the magnetic particles in the ferrofluid, which, in turn, causes the domains to align along the field. Thus the 363-angstrom spacing between the viruses is perpendicular to the field and produces diffraction peaks only in that direction.

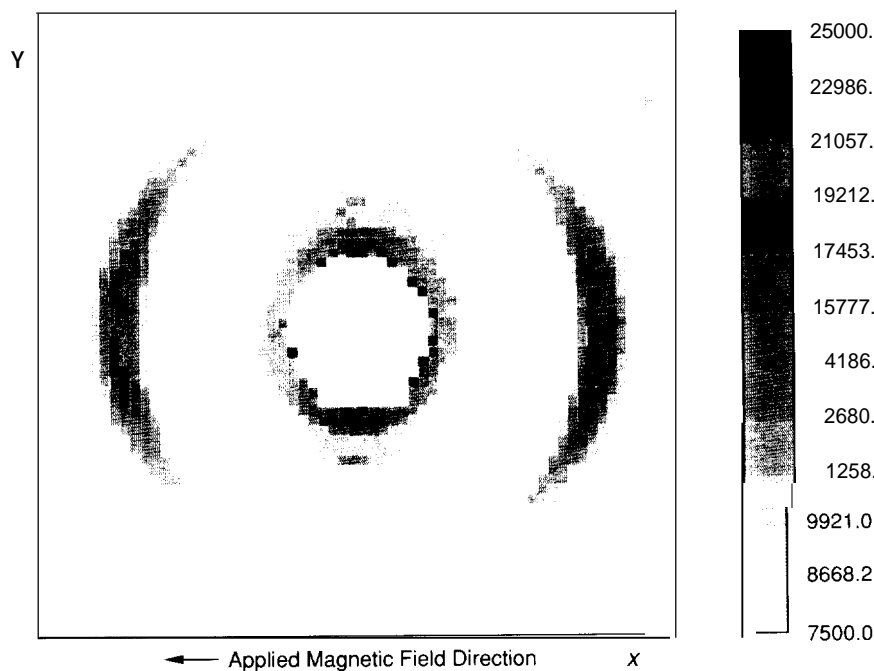


another diffraction peak should be seen, one produced by the 23-angstrom distance between the turns of the helical viral coat protein (see Fig. 1). That peak should occur at scattering angles much greater than those included in the data of Fig. 3 and at a direction perpendicular to the direction at which the diffraction peak due to the packing distance is observed. We moved the detector closer to the sample and did indeed see a diffraction peak corresponding to a

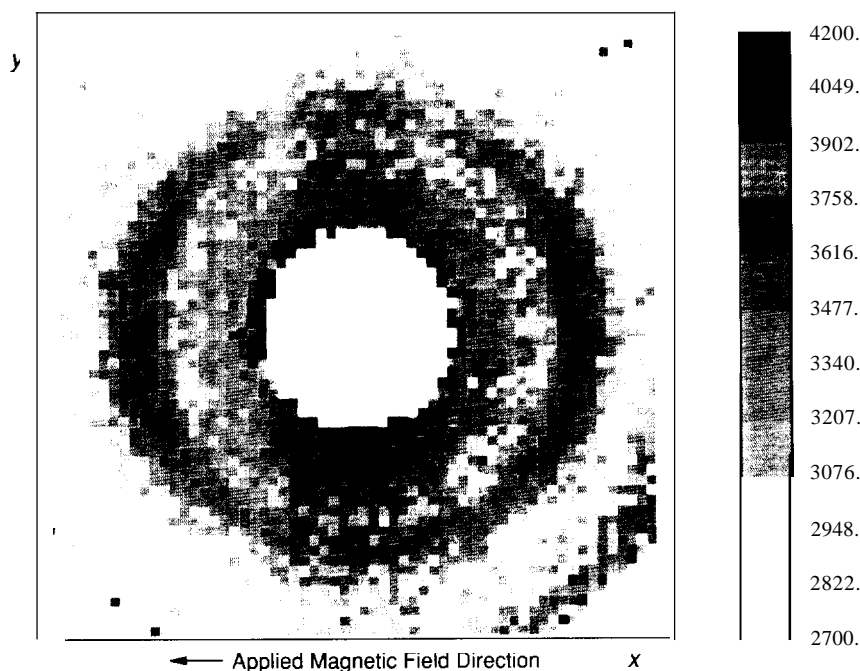
23-angstrom helical pitch (Fig. 5a). The existence of the peak confirms the structural integrity of the viruses in the ferrofluid, and its orientation parallel to the magnetic field is consistent with alignment of the long axes of the viruses parallel to the magnetic field (Fig. 4b).

Although our experiments demonstrated alignment, we wondered whether it was caused by intrinsic magnetic properties of TMV or by one of the mechanisms depicted in Fig. 2. The

(a) Diffraction Peak at 23 angstroms



(b) Diffraction Peak at 25 angstroms



NEUTRON DIFFRACTION PEAKS FROM TMV AND TRV

Fig. 5. Color-coded intensity data for (a) tobacco mosaic virus and (b) tobacco rattle virus dispersed in a ferrofluid in the presence of a 0.3-tesla applied magnetic field. Note the diffraction peaks at 23 and 25 angstroms, which correspond to the regular spacing between the turns of the helical protein coats of TMV and TRV, respectively. The diffraction peaks appear only in the x direction, that is, along the direction of the applied field. Thus, the ferrofluid has aligned the viruses with their long axes along the applied magnetic field.

trated samples of TRV did not align in a normal solution even in a high applied magnetic field (7 teslas). However, when TRV was dispersed in the ferrofluid, a field of 0.5 tesla was sufficient to produce alignment comparable to that obtained for TMV in phosphate buffer, Figure 5b shows a diffraction peak corresponding to the 25-angstrom pitch of the helical TRV protein coat, similar to the peak obtained for TMV in Fig. 5a. The position of the peak indicates that the viruses are aligned parallel to the applied magnetic field. Moreover, like TMV in phosphate buffer, TRV showed no evidence of any domains.

Our preliminary studies demonstrated that the ferrofluid technique can orient elongated biological assemblies irrespective of their intrinsic magnetic properties and without disrupting their structural integrity. Furthermore, the orientation is sufficiently great to facilitate measurement of internal structural parameters. Future studies will concentrate on increasing the degree of alignment so that higher-resolution data can be obtained. ■

viruses could have been ordered by diamagnetic moments; however, the magnetic field strength was smaller than is generally needed to achieve the degree of alignment indicated by the data. Such alignment often requires the presence of ordered domains in the sample. So we repeated the experiments, this time adding phosphate buffer, the ions of which are known to disrupt domain structure. Thus alignment based on the intrinsic diamagnetism of the viruses is

not expected in the presence of phosphate buffer. We found that the viruses were still aligned but to a lesser degree. That result suggests not only that the ordering is due to the presence of the ferrofluid but also that the mechanism depicted in Fig. 2b may enhance the alignment of TMV.

The tobacco rattle virus is genetically unrelated but morphologically quite similar to TMV. TRV shows only poor orientation under shear, and concen-