References and Notes

1. J. P. Keene, *J. Sci. Instr.* **41**, 493 (1964).
2. E. Hart and J. W. Boag, *J. Amer. Chem. Soc.* **84**, 4090 (1962); I. A. Taub, M. C. Sauer, L. M. Dorfman, *Discussions Faraday Soc.* **36**, 206 (1964).
3. G. E. Adams, J. H. Baxendale, J. W. Boag, *Proc. Roy. Soc. Ser. A* **277**, 549 (1964).
4. J. A. Bell and H. Linschitz, *J. Amer. Chem. Soc.* **85**, 528 (1963).
5. G. Porter and M. W. Windsor, *Proc. Roy. Soc. Ser. A* **245**, 238 (1958).
6. H. Carter, B. J. McClelland, E. Warhurst, *Trans. Faraday Soc.* **56**, 458 (1960).
7. S. Arai and L. M. Dorfman, *J. Chem. Phys.* **41**, 2190 (1964).

† Present address: Chemistry Division, Argonne National Laboratory, 9700 Cass Avenue, Argonne, Illinois 60440.

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Rotation Technique in Electron Microscopy of Viruses

Abstract. *Some of the features revealed by the rotation technique of examining electron micrographs may be artifacts. To avoid misinterpretation, "rotated" photographs should be compared with originals. The criterion of reinforcement of structures in the "rotated" photographs is not enough, and it cannot be presumed that all the structures reinforced are actually present in the object under study. Further tests for radial symmetry in the arrangement of elements in a pattern should be made.*

Markham *et al.* (*1*) described some methods for increasing the amount of information that can be obtained from electron micrographs of virus particles. Utilizing the symmetry of the virus particle, these workers devised a rotation technique which, they suggested, could provide an increase in resolution above that which is on the original plate. The apparatus used is quite simple and consists of a circular card with a pin through its center. The periphery of the card is divided into an integral number of equal angular divisions, each corresponding to a simple fraction of one complete circle. The pin is fixed on a piece of board, and the whole card is capable of rotation around the pin. The object to be examined is enlarged photographically to a convenient size and is projected onto the card and centered as well as possible. A piece of photographic paper or a lantern slide is then attached to the card. An exposure of $1/n$ times the exposure required for the photographic material, where n is the number of subdivisions to be used, is given. The card is then rotated by one division, or $360°/n$, and another exposure given. This is continued until n exposures have been made. The paper or the plate is then developed. If n is the fundamental periodicity in the specimen, and if the centering is accurate, this periodicity will appear to be reinforced. A departure from this relationship will result in a meaningless blur. Once a reinforcement is achieved, tests may be performed to see whether it is a real one, by making successive exposures in which n is replaced by $n\text{-}x$ or n/x, where x is an integral number. Naturally, any flaw in the object or photograph is repeated n times. Markham *et al.* (*1*) suggested that the foregoing test will serve to detect such artifacts. Although they stressed that the technique should be applied with caution, the artifacts inherent in such a technique have not been clearly pointed out and discussed.

To safeguard against a possible misinterpretation of such rotated micrographs, we describe here some of our observations.

Several authors have already used the rotation technique in studying the structure of some viruses (*1–3*). The technique has also been used in studies on the structure of other symmetrical objects (*4*). In some of these instances, only the rotated micrographs are presented, and it is not clear whether a comparison was made between the rotated and unrotated photographs and whether the photographs were found to be in agreement. In our opinion, such a comparison is necessary to eliminate the possibility of misinterpretations attributable to artifacts (*2*).

By rotating through n successive positions (where n is an integer) and superimposing all n, one may very likely detect a structure with n "lobes," even if the original has no such lobes. If so, the degree of symmetry of this structure is not a direct indication of the symmetry of a corresponding structure in the original. The criterion of reinforcement alone is not enough and it cannot be presumed that all the structures appearing reinforced are actually present in the object under study. This is illustrated by Figs. 1, 2, and 3.

An arrangement of random points, taken from MacKay (*5*), is shown in Fig. 1*A*. The same diagram is shown in Fig. 1, *B* through *F*, after rotating 2/2, 3/3, 4/4, 5/5, and 6/6, respectively. The numbers indicate the degree of rotation (that is, 2/2 is $360°/2 = 180°$, 3/3 is $360°/3 = 120°$, and so on) and the number of exposures made (2, 3, 4, 5, and 6 represent directly the number of exposures corresponding to

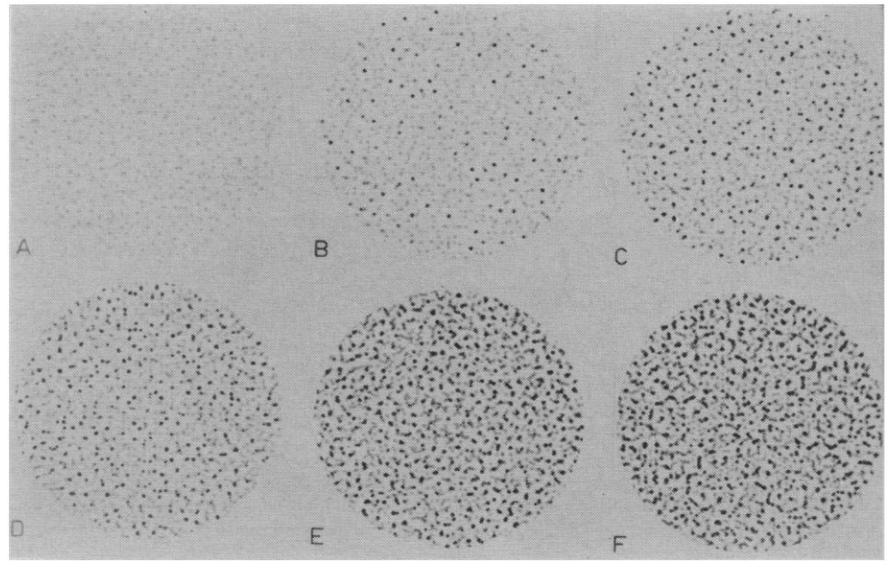

Fig. 1. (*A*) A pattern with random points (*5*). (*B* through *F*) The same pattern photographed by the rotation method after rotating 2/2, 3/3, 4/4, 5/5, and 6/6, respectively. The denominator indicates the number of divisions of rotation (or degrees—that is, $360°/n$ where $n = 2, 3, 4, 5,$ or 6). The numerator shows the number of exposures made corresponding to the degree of rotation.

the degree of rotation). A reinforcement of some of the points can be clearly seen in these photographs. However, no really definite patterns can be seen in any of these photographs except Fig. 1, *E* and *F*, where five and six localized patterns, an indication of five- and sixfold multiplicity, respectively, can be distinguished around the center. All these patterns are quite artificial and cannot be identified on the unrotated diagram (Fig. 1*A*).

Figure 2*A* shows a pattern of elements possessing three-, four-, and fivefold radial symmetries. Some of the elements have been omitted at random, while other points have been added randomly. This procedure crudely represents loss of information and development of "background noise" which inevitably accompany the processing of a specimen for examination, and may be said to be closer to the practical situation than a distribution of purely random points, either alone, or combined with a complete pattern of elements exhibiting radial symmetry. The pattern after rotating 2/2, 3/3, 4/4, 5/5, and 6/6 is shown in Fig. 2, *B* through *F*. The differences between these photographs are quite obvious. Not only are the different elements of symmetry in Fig. 2*A* reinforced in the rotated photographs, but they appear in larger numbers—points are synthesized for the randomly omitted elements. Also, some points appear reinforced solely because of random reinforcement, and in the absence of prior knowledge and comparison with the unrotated diagram, it is not easy to say whether all the reinforced points in, say, Fig. 2*F*, represent a symmetrical distribution of elements in the original.

In Fig. 3*A* is shown a model built up of 32 table tennis balls representing the capsomeres in a virus particle. Such a model has been used to illustrate the structure of the protein shell of turnip yellow mosaic virus (*2*, *6*). The model is constructed according to 5:3:2 symmetry and is shown in Fig. 3*A* in the fivefold symmetry position. Parts *B*–*F* in Fig. 3 are photographs made after rotating 2/2, 3/3, 6/6, 8/8, and 10/10, respectively. Complications in interpretation can easily arise if the unrotated photograph is not used as a basis for comparison and as a control. The symmetrical subdivision of the subunits surrounding the central one, because of the rotation and consequent redistribution of the structures, can also

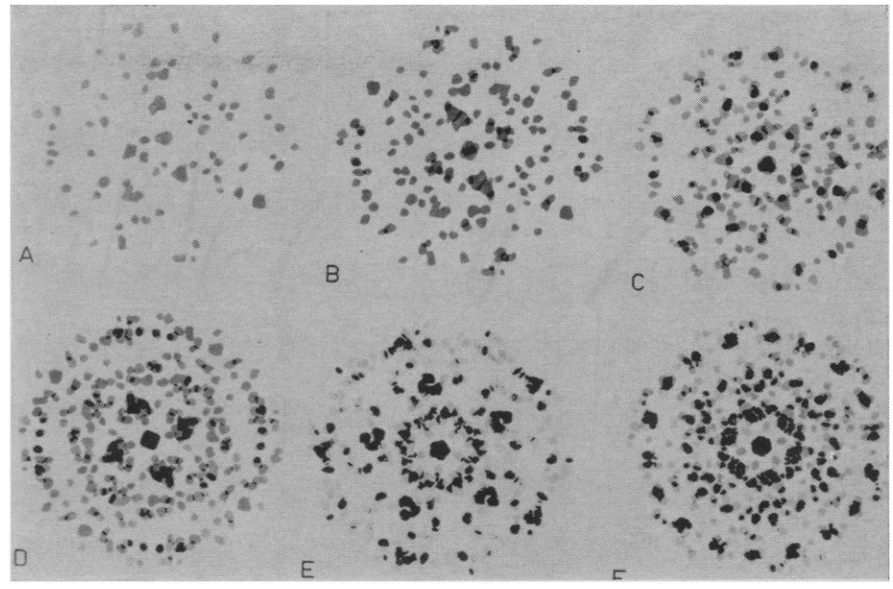

Fig. 2. (*A*) A pattern in which there are random points together with elements having three-, four-, and fivefold symmetry. (*B* through *F*) The same pattern photographed by the rotation method after rotating 2/2, 3/3, 4/4, 5/5, and 6/6, respectively.

introduce additional problems of interpretation (Fig. 3, *B* and *F*: these figures are identical owing to the fivefold symmetry in the original).

Two further tests for radial symmetry in the arrangement of elements in a pattern are suggested here.

1) If a pattern is rotated x/n revolutions (where x and n are integers and have no common factor other than unity) and this new orientation is superimposed on the original *once only*, then it may be possible to detect a structure with Tn lobes, where T is also an integer. If this is so, then the degree of symmetry of this structure is a direct indication of n-fold symmetry in the original, and suggests the presence of Tn-fold symmetry. To confirm the presence of Tn-fold symmetry, it is

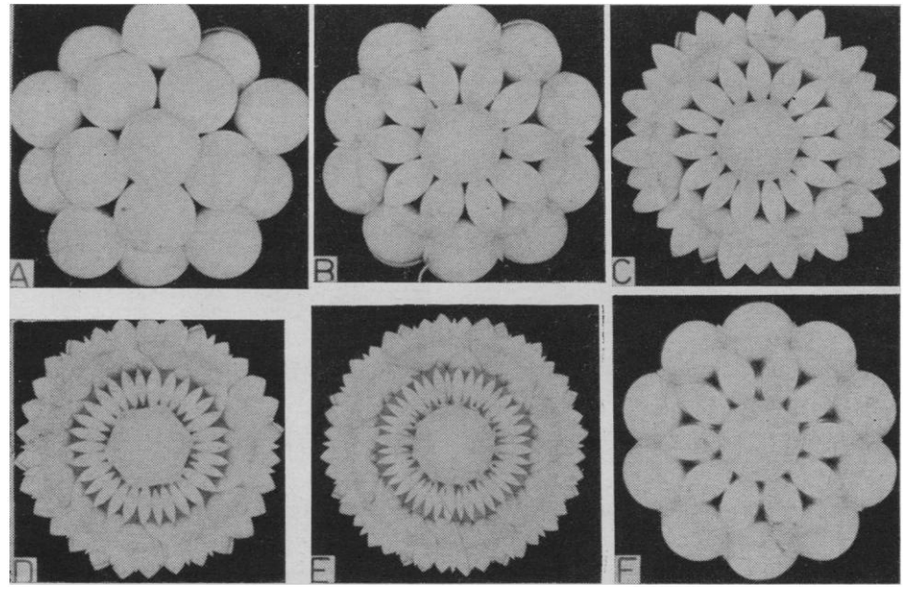

Fig. 3. (*A*) A model with 32 table tennis balls arranged according to 5:3:2 symmetry, shown in fivefold symmetry. (*B* through *F*) The same model photographed by the rotation method after rotating 2/2, 3/3, 6/6, 8/8, and 10/10, respectively. A 5/5 rotation gives a picture similar to the unrotated original (*A*); (*B*) and (*F*) are identical because of the fivefold symmetry in the original.

necessary to repeat the procedure, but with a rotation of x/Tn.

2) Where a pattern has been rotated x/n revolutions, the resulting n-fold symmetrical figure can be used to define *potential* axes of *mirror* symmetry in the original. Having obtained these axes and determined their location on the original by comparison with reference marks drawn on the original, which also serve to define the center of rotation, one can compare the resulting mirror-superpositions to evaluate the significance of the symmetrical distribution of structures suggested by the rotation technique.

It is suggested on the basis of these photographs that the criterion of reinforcement alone, as suggested by Markham *et al.* (*1*), is not sufficient in interpreting structures appearing in rotated micrographs. It is also clear that the only true test to check whether the reinforcement is real or not, or whether it has real meaning, is to compare the structures detected in the rotated micrographs with those in the original. This in turn means that the use of the rotation technique is restricted to enhancing structures which are already visible, or at least have a known symmetry and a visible center of symmetry, in the unrotated micrographs. With this restriction, the technique can be of considerable value.

Finally, it should be pointed out that the criterion of *biological* significance is not the probability that symmetry is present in the original photograph, but the improbability that such symmetry would have turned up by chance if the biological structure were not symmetrical. To test this ideally, one has to compare the results of similar procedures on a whole series of biologically similar specimens.

HARI OM AGRAWAL*
Department of Virology, State Agricultural University, Wageningen, Holland

J. W. KENT†
Weed Control Department, Institute for Biological and Chemical Research on Field Crops and Herbage, Wageningen, Holland

D. M. MACKAY
Department of Communication, University of Keele, Staffordshire, England

References and Notes

1. R. Markham, S. Frey, G. J. Hills, *Virology* **20**, 88 (1963).
2. H. O. Agrawal, *Neth. J. Plant Pathol.*, in press.
3. ———, *Mededel. Landbouwhogeschool Wageningen* **64–5**, 53 (1964); ———, in preparation; C. Vasquez and P. Tournier, *Virology* **24**, 128 (1964).
4. J. A. Lucy and A. M. Glauert, *J. Mol. Biol.* **8**, 727 (1964); M. C. Ledbetter and K. R. Porter, *Science* **144**, 872 (1964).
5. D. M. MacKay, *Nature* **203**, 993 (1964).
6. H. E. Huxley and G. Zubay, *J. Mol. Biol.* **2**, 189 (1960); H. L. Nixon and A. J. Gibbs, *ibid.*, p. 197.
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* Present address: Virus Laboratory, University of California, Berkeley.
† Present address: Department of Biology, University of Keele, Staffordshire, England.

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Linkage in Control of Allotypic Specificities on Two Different γG-Immunoglobulins

Abstract. *Allotypic specificities were identified on two different myeloma proteins and the corresponding normal γG-immunoglobulins of BALB/c mice. When the sera of F_2 progeny from a cross between BALB/c mice having the a1 allotype and AL mice having the a4 allotype were tested for these specificities, it was found that the two allotypic specificities of the BALB/c mice were either both present (87 mice) or both absent (36 mice), an indication of linkage in their genetic control.*

Isoantibodies to γG-immunoglobulins (*1*, *2*) are produced in mice of selected inbred strains immunized with immunoglobulins of other inbred strains (*3*, *4*). By reaction with antibodies from various donor-recipient combinations, five allotypic (antigenic) specificities a1, a2, a3, a4, and a5 were found among 38 inbred strains (*5*, *6*). Each inbred strain exhibited only one of these allotypic specificities (*5*). When an inbred mouse with one allotype is mated to an inbred mouse with another allotype, the F_1 hybrids have both allotypes. In the F_2 progeny, the mice have either one or both allotypes. The tests of F_2 progeny derived from mice with different allotypes indicated that the five allotypic specificities are controlled by five alleles at a single genetic locus (designated *Asa*). That these allotypic specificities of the *Asa* locus are associated with γG-immunoglobulins was based upon immunoelectrophoretic experiments which showed precipitin arcs characteristic of these proteins (*5*). The heritably controlled synthesis of specific molecular types of immunoglobulins by different transplantable plasma-cell tumors in the inbred a1-BALB/c strain of mice has provided a source of specific immunoglobulins of the light (L) chain, γA, γF, γG-Be1 and γG-Be2 types (*7–9*); these can be tested individually for the presence of the a1 allotypic specificity. Fahey, Wunderlich, and Mishell (*2*) reported an antiserum to the a1 allotype that reacted with the γG-Be1 (γ_2a) but not with the γG-Be2 (γ_2b) mouse myeloma protein.

We have now found that antisera to allotypes could be obtained which were specific for two different γG-immunoglobulins in BALB/c mice, that is, one antiserum reacted with γG-Be1, the other with γG-Be2. In addition, genetic data are presented which show that the two allotypic specificities present on distinct γG-immunoglobulins within the same strain are inherited as if controlled at the same genetic locus.

Of the two antisera to allotypes, the one which reacted specifically with one myeloma type γG-immunoglobulin (γG-Be1) of a1-BALB/cAnN mice is designated anti-a1′, and it was obtained from a2-SM/J mice immunized with immunoglobulins of a1-DD/He mice (*6*, *10*). The other, designated anti-a1″, reacted specifically with another myeloma type γG-immunoglobulin (γG-Be2) of a1-BALB/c mice was obtained from inbred a4-AL/N mice immunized with immunoglobulins of a1-C58/J mice. The specificity of these antisera was determined by reaction with three γG-Be1 mouse myeloma proteins (designated Adj. PC-5, LPC-1, and MOPC-173) and three γG-Be2 mouse myeloma proteins (designated MOPC-141, MOPC-172, and MOPC-184) (*9*). These myeloma proteins were isolated by ammonium sulfate precipitation and diethylaminoethyl (DEAE) chromatography from sera of BALB/c mice bearing transplantable plasma cell tumors (*9*). The anti-a1′ reacts with the γG-Be1 (Adj. PC-5) but not with the γG-Be2 (MOPC-141) myeloma protein (Fig. 1). The anti-a1″ reacts with the γG-Be2 (MOPC-141) but not with the γG-Be1 (Adj. PC-5). Neither antiserum reacted with five γF myeloma proteins, MOPC-31C (Fig. 1), MOPC-70A, MOPC-21,