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

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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,

MOPC-28 and Adj. PC-23, nor with ten light chain and three γA myeloma immunoglobulins.

Among 123 F_2 progeny of the cross between a1-BALB/cAnN and a4-AL/N mice, there are 28 a^1a^1 homozygotes, 59 a^1a^4 heterozygotes, and 36 a^4a^4 homozygotes (*5*); a distribution consistent with the hypothesis that a^1 and a^4 are alleles ($p \gtrless .5$ to .7). The antiserum to a1 (from a4 anti-a1 mice) used in the previous study (*5*) gave precipitin reactions with both γG-Be1 and γG-Be2; however, when absorbed with γG-Be1 (Adj. PC-5) it (the absorbed serum) reacted only with γG-Be2 (such as MOPC-141 in Fig. 1) and was designated anti-a1″. With the availability of the anti-a1″ specific for γG-Be2 and the anti-a1′ specific for γG-Be1, it was of interest to test the same F_2 progeny with these more specific antisera since this would give information concerning independent assortment and linkage. The serum of each mouse of the total (123) F_2 progeny either gave precipitin lines with both antisera or did not react with either antiserum; 51 representative examples (Nos. 706–756) are shown in Fig. 1. These results were confirmed by another test with 94 F_2 offspring from the cross of a1-BALB/cAnN and a2-C57BL/6N mice; the anti-a1′ and the anti-a1″ gave precipitin reactions with the sera of the same mice.

These results indicate that two different allotypic specificities (a1′ and a1″) present on different γG-immunoglobulins are inherited as though controlled at a single genetic locus. This result is in accord with that shown for the rabbit where two allotypic specificities on two different γG-immunoglobulins were also shown to be controlled as if by a single genetic locus (*11*). However, in the rabbit, neither the two immunoglobulins nor the two specific antisera were obtained separately.

The lack of precipitin reactions with light chain, γA and γF, myeloma immunoglobulins suggests that the allotypic specificities are not on the light chains but on the heavy chains. Mishell and Fahey (*12*) showed that antisera to allotypes reacted with the papain digested Fc-fragment (*1*) which is a part of the heavy chain. We have confirmed this finding with the Fc-fragment of γG-Be1 (MOPC-173) (*13*).

Tryptic peptide maps of the reduced and alkylated heavy chains of γG-Be1 and γG-Be2 myeloma immunoglobulins indicated differences in amino acid sequences (*9*), an implication of genetic control by two structural genes. If the two allotypic specificities depend on differences in amino acid sequence as had been shown for the allotypic specificities on light chains in the rabbit (*14*), the interpretation could be made that *Asa* locus contains the structural genes for the heavy γG-Be1 and γG-Be2 chain. However, genes could control the allotypic specificities on these proteins by means other than amino acid sequence, such as by carbohydrate prosthetic groups. If two closely linked

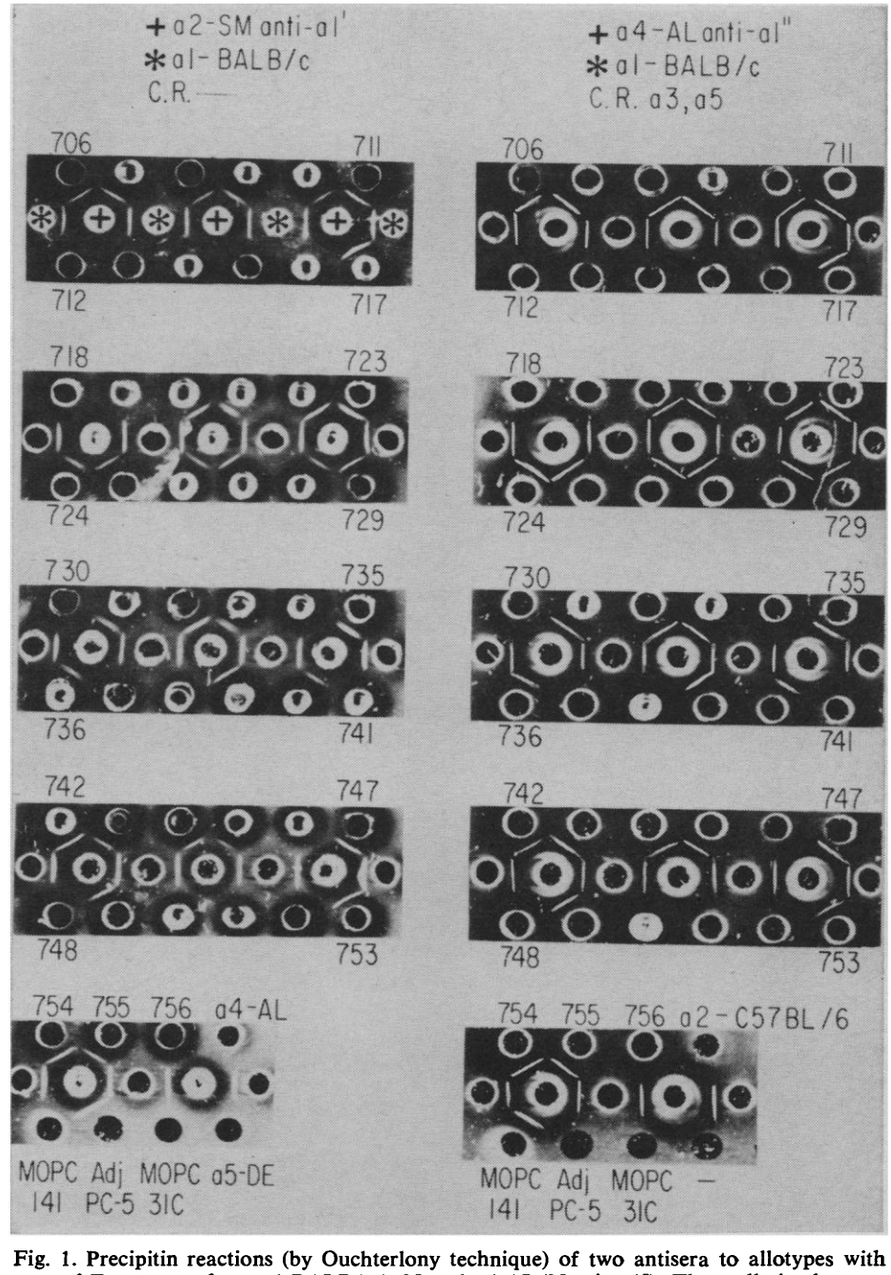

Fig. 1. Precipitin reactions (by Ouchterlony technique) of two antisera to allotypes with sera of F_2 progeny from a1-BALB/cAnN and a4-AL/N mice (*5*). The wells in the center row of each design were filled alternately with a1-BALB/c reference serum (*) and either anti-a1′ (+) as shown in the left column or anti-a1″ (+) as shown in the right column. Sera from individual F_2 mice (Nos. 706-756) were placed in the wells of the upper and bottom rows as indicated. Myeloma proteins and sera of selected inbred strains as controls were placed in the wells indicated in the smaller diagrams at the bottom of each column. The mouse strains and their allotypes are indicated. Cross-reactions (C.R.) of antibodies to allotypes with allotypes other than that of the donor strain are indicated. The a2-SM anti-a1′ (γG-Be1) was prepared by absorption of anti-a1 from a2-SM mice with γG-Be2 immunoglobulin (MOPC-141). The a4-AL anti-a1″ (γG-Be2) was prepared by absorption of anti-a1 from a4-AL mice with γG-Be1 immunoglobulin (Adj. PC-5).

structural genes do control the heavy γG-Be1 and γG-Be2 chains, it may be possible to detect recombinants among the backcross progeny of F_1 (BALB/c × C57BL/6) mice to C57BL/6 mice.

Herzenberg (*15*) recently reported linkage of allotypic specificities for γA (β_2A) and γG-Be1 (γ_2a) immunoglobulins. The tryptic-peptide maps of the γA and γG-Be1 heavy chains from γA and γG-Be1 myeloma immunoglobulins show vastly different patterns (*8*, *9*). Thus there appear to be three closely linked allotypic specificities at the *Asa* locus. These findings indicate that at least one complex locus takes part in the synthesis of heavy-chain subunits of immunoglobulins in mice.

The allotypic specificities of immunoglobulins in mice are the most accessible of any mammalian system for combined chemical and genetic analysis and provide useful comparative models for the analysis of data on the genetic control of human immunoglobulins where questions of linkage are more difficult to establish (*16*).

ROSE LIEBERMAN
SHELDON DRAY
MICHAEL POTTER
Laboratory of Clinical Investigations and Laboratory of Immunology, National Institute of Allergy and Infectious Diseases and Laboratory of Biology, National Cancer Institute, Bethesda, Maryland

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1. A "Nomenclature for Human Immunoglobulins" recommended by the World Health Organization Committee is applied here to the mouse immunoglobulins: γA (for β_2A or γ_1A); γM (for β_2M or γ_1M) and γG (for 7*S* γ or γ_2). We have further designated the three 7*S* γG-immunoglobulins as γF, γG-Be1 (Bethesda 1) and γG-Be2 (Bethesda 2) based on peptide maps of the respective myeloma heavy polypeptide chains; this notation corresponds to γ_1, γ_2a, and γ_2b characterized by Fahey, Wunderlich, and Mishell (*2*) on the basis of immunochemical differences. The Fc-fragment obtained by papain digestion is the nonantigen binding fragment (corresponding to the crystallizable fragment in rabbit antiserum). R. Ceppelini *et al.*, *Bull. World Health Org.* **30**, 447 (1964).
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5-Hydroxytryptamine in Single Neoplastic Mast Cells: A Microscopic Spectrofluorometric Study

Abstract. *5-Hydroxytryptamine, catecholamines, and histamine in mast cells can be distinguished by fluorescence microscopy. Microscopic spectrofluorometry can be used to estimate 5-hydroxytryptamine in single neoplastic mast cells grown in vitro, and to study the effect of reserpine on these cells.*

Treatment of various tissues with formaldehyde vapor produces fluorescent derivatives of a number of amines which have characteristic colors when examined by fluorescence microscopy. Lagunoff, Phillips, and Benditt (*1*) treated freeze-dried tissues with formaldehyde vapor to demonstrate histamine in mast cells. Falck and coworkers (*2*) treated freeze-dried tissues with formaldehyde vapor to demonstrate norepinephrine, epinephrine, and dopamine in brain and in peripheral adrenergic fibers, and these investigators attributed yellow-green fluorescence to norepinephrine, and yellow fluorescence to 5-hydroxytryptamine (5HT). Adams-Ray *et al.* (*3*) recently applied this histochemical technique to mast cells in the skin of mammals, and found in some the greenish-yellow color associated with catecholamines (*4*).

The treatment of various tissues with formaldehyde vapor thus appears to result in the formation of fluorescent derivatives of various amines (*5*). Since it may be difficult to differentiate among these derivatives visually by fluorescence microscopy, an objective histochemical technique is required. Several investigators have used microscopic fluorometry or spectrofluorometry to estimate low concentrations of endogenous compounds in tissues or single cells (*6*). Ritzén (*7*) described the application of microscopic spectrofluorometry to the identification of formaldehyde derivatives of norepinephrine and 5HT in various tissues.

To select excitation and emission wavelengths which would permit separation of the three major categories of biogenic amines associated with mast cells, the following determinations were made. The fluorescence characteristics of 5HT, norepinephrine, and histamine were determined by incubating 1.0 m*M* solutions of each amine in 20 m*M* phosphate buffer (*p*H 6.8) containing 1 percent formaldehyde, at 60°C for 2 to 4 hours. Excitation and emission spectra were determined in the Aminco-Bowman spectrofluorometer and corrected for phototube response (RCA 1P21) with the manufacturer's average spectral response curve. Fluorescence characteristics in the solid state were determined by drying 0.01 ml of 5 m*M* amine in 1 percent crystalline ovalbumin solution on a microscope slide, heating the residue in the presence of paraformaldehyde (polymerized formaldehyde) at 80°C for 1 hour, and examining the material in the microspectrofluorometer (*8*). The results obtained are summarized in Table 1. Values for norepinephrine in solution varied widely with *p*H, but in the solid state the emission maximum was very close to the value reported by Corrodi and Hillarp (*5*). The fluorescence of the 5HT derivative did not vary in the aqueous and dry states, and was close to that previously reported (*5*). The histamine derivative showed an increase in excitation and emission maxima in the solid state, in comparison with aqueous solution; these values are similar to those obtained by Lagunoff (*9*) under somewhat different conditions.