Large clumps that appear light colored in the upper center of both c and d in Fig. 1 were actually dull orange, and bacterial cells could be distinguished on the margins of these clumps. There is a further improvement in the microscopic appearance of the preparations in the background of fields devoid of soil particles (Fig. 1c, upper left). Such areas were nearly black with no indication that either conjugate was retained by soil films. Perhaps some films wash free as a result of the gelatin treatment.

Results of treatment with conjugates of varying ratios of dye to gelatin are summarized in Table 1. Amounts of rhodamine isothiocyanate ranging from 0 to 120 μg per milligram of gelatin were added to a constant amount of 2 percent autoclaved gelatin, *p*H 10. Conjugated fractions were analyzed on a Klett colorimeter (green filter) for dye content after gel filtration. The standard curve was based on concentrations of RhITC at 0.4 to 40.0 mg per 100 ml in 2 percent gelatin at *p*H 10. Protein content of each conjugated fraction was determined by the Kjeldahl method (*5*). Staining properties were checked on slides recovered from sterilized soil inoculated with *R. japonicum*, and the various gelatin preparations were overlaid with the same batch of *R. japonicum* FITC conjugate. The unconjugated (zero rhodamine) gelatin was prepared with dialysis instead of gel filtration. This preparation may be especially useful for detecting a certain microorganism in the soil without reference to its relations with soil particles. The background was very dark, and specifically stained bacteria could be seen clearly, but soil particles were barely discernible. Mixtures with less than 20 μg of RhITC per milligram of gelatin were generally most satisfactory. Conjugates made with 8 μg of gelatin were used routinely.

The effectiveness of the gelatin-RhITC conjugate in blocking nonspecific adsorption was dependent on the *p*H of the gelatin at conjugation. There was conjugation at *p*H 3, 5, 7, and 8.5, with best conjugation at *p*H 7.0; none of these conjugates prevented nonspecific staining. The only effective gelatin-RhITC conjugates were those which reacted at *p*H 10.

Slides prepared with gelatin-RhITC conjugate were suitable for FA examination for at least 1 week. Longer periods of storage resulted in changes in the fluorescence of the bacteria from yellow-green to orange-yellow. Similar effects were noted when specific conjugate was not added to the gelatin film within a few hours after drying.

Conjugates of gelatin and lissamine rhodamine B were as satisfactory as the gelatin-RhITC conjugates. Lissamine rhodamine B is cheaper but must be converted to the sulfonyl chloride (*7*) before being conjugated. Conjugates prepared with tetramethyl rhodamine isothiocyanate were unsatisfactory over a range of ratios of dye to protein. The red fluorescence of the background was too intense to provide contrast.

Only exploratory studies on the possible use of gelatin preparations for clinical applications were made. Results suggest that gelatin treatment probably can be useful in controlling nonspecific staining for at least some diagnostic problems. Smears of homogenized normal mouse spleen, liver, heart, or lung tissues, impression slides of human spleen, and frozen sections of human lung adsorbed the gelatin-RhITC conjugate, resulting in orange-brown background fluorescence. Nonspecific adsorption of *Rhizobium*-FITC conjugated antiserum was minimum when added to the gelatin-treated preparations. Without gelatin treatment each tissue demonstrated substantial nonspecific staining by the FITC conjugate. Similar tests with deparaffinized sections of human liver and smears of human bone marrow, however, demonstrated little or no decrease in nonspecific staining as a result of gelatin-RhITC treatment.

In further tests (*8*), frozen impression smears of liver and spleen from mice experimentally infected with *Pasteurella tularensis* took up the gelatin-RhITC both in and around tissue cells. Specific staining with *P. tularensis*-FITC conjugate (1 : 8 dilution) was not impaired by the gelatin treatment. Treatment of impressions of infected mouse spleen with either undiluted specific FITC conjugate or undiluted FITC conjugate from normal rabbit resulted in pronounced nonspecific staining. Virtually all of this nonspecific staining was blocked when the undiluted FITC conjugates were applied after gelatin-RhITC treatment. The *P. tularensis* cells showed 4 + fluorescence with the specific staining system despite gelatin treatment and could be seen more clearly and distinctly than on corresponding slides without gelatin. Equally marked benefits were obtained from gelatin-RhITC treatment of paraffin sections of human lymph nodes (from a patient infected with *P. tularensis*). Nonspecific staining was not blocked by gelatin treatment in tests with monolayers of KB mammalian cells (*2*), or with leukocytes in buffy-coat preparations.

The gelatin preparations tested on animal tissues were those developed for use on soil. These preparations provided excellent control of nonspecific staining for soil materials and appeared promising for various tissue preparations as well.

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MN Blood-Group Locus: Data Concerning the Possible Chromosomal Location

Abstract. *Combined data derived from clinical, cytogenetic, and blood-grouping studies of one family suggest that the* MN *locus is on the long arm of either the No. 2 or the No. 4 chromosome.*

Data accumulated during the investigation of a family brought to our attention through a developmentally abnormal child are briefly summarized here as a contribution toward the genetic mapping of the human chromosomes (*1*).

A 30-month-old child with mental retardation, a small unusually shaped head, facial dysmorphy, unusual dermatoglyphics, and an abnormal gait has been found by cytogenetic and autoradiographic analysis of 50 dividing blood lymphocytes to have an abnormal chromosomal complement (Fig. 1). A long distal segment of the long arm of a No. 2 chromosome has been translocated to a point very near the distal tip of the long arm of a No. 4 chromosome. Consequently, the abnormal No. 4 chromosome has an elongated long arm, and the abnormal No. 2 resembles members of group C(6-X-12) morphologically. This rearranged complement of chromosomes may be described as 46,XY,t(2q−4q+), according to the nomenclature of the Chicago Conference (*2*). The pattern of DNA synthesis in the longer of the two abnormal chromosomes, the No. 4q+, was examined with autoradiography after labeling with tritiated thymidine. The normal No. 4 is known to undergo extensive DNA synthesis later in the cell cycle than most autosomal chromatin (*3*). In contrast, a long distal segment of the long arm of the No. 2 normally completes essentially all synthesis relatively early. The translocation here has joined these two segments which differ greatly in their patterns of synthesis, and yet each segment was found to have maintained in large part its established pattern. The length of late-synthesizing chromatin in the No. 4q+ chromosome appears similar to that in the unaffected No. 4. The distal portion of the long arm of a No. 2 now in its new position adjoining the late-synthesizing long arm of a No. 4 still completes its synthesis relatively early, remaining in synchrony with its homologous segment in the unaffected No. 2.

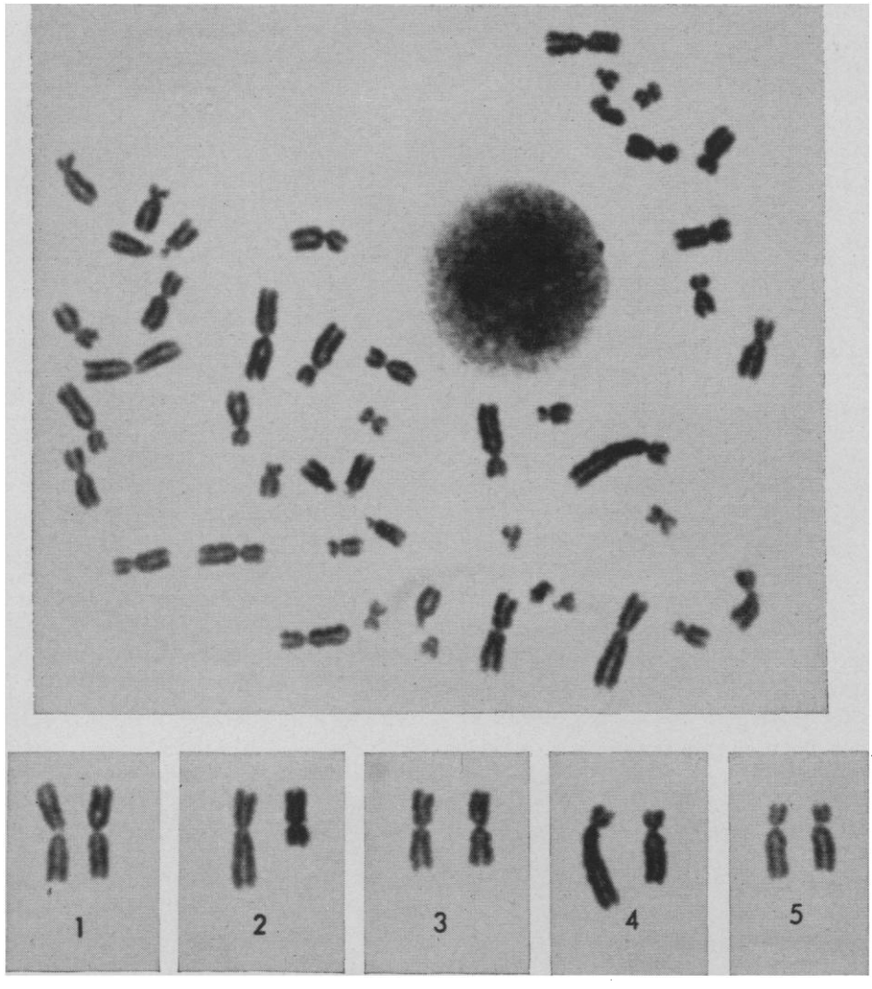

Fig. 1. A dividing lymphocyte from the propositus is shown in upper part of the figure. In the lower part, the chromosomes of group A(1–3) and group B(4–5) have been paired. A rearrangement has occurred affecting the long arms of a No. 2 and a No. 4 chromosome. Orcein stain (× 2000).

Typing of red blood cells shows that the propositus is N-negative, s-negative (type MS) while the father is M-negative, S-negative (type Ns). There is important evidence from dosage tests (*1*) that the propositus has only one *MN* gene. He gave single-dose reactions with anti-M, which showed that he is not homozygous for *M*. The father, however, gave double-dose reactions with anti-N, confirming that he is homozygous (*N.N*). The mother and both sibs of the propositus gave single-dose reactions with anti-M and with anti-N. There is no evidence of nonpaternity from the tests of 14 other loci, and the Y chromosome of the propositus, which is slightly shorter than that usually observed, resembles those of his father and brother.

It appears that in the propositus a paternal *N* gene has been either lost or inactivated during chromosomal rearrangement. That the child is phenotypically abnormal implies that some genetic imbalance did result from the chromosomal rearrangement. We cannot specify the exact mechanism by which he has become hemizygous at the *MN* locus, or has a condition that cannot be distinguished from hemizygosity by serological methods. The evidence suggests, however, that the *MN* locus is either in the middle of the long arm of chromosome No. 2 or near the distal end of the long arm of chromosome No. 4.

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2. D. Bergsma, Ed., *Chicago Conference: Standardization in Human Cytogenetics* (The National Foundation, New York, 1966). Birth Defects: Original Article Series, vol. 2, No. 2. Abbreviations: t is translocation affecting the chromosomal regions set off parenthetically; q, the long arm of a chromosome; —, a decrease in length; and +, an increase in length.
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