Edited by Robert S. Krooth, M.D.

Somatic Cell Genetics

FOURTH MACY CONFERENCE ON GENETICS

SOMATIC CELL GENETICS

Fourth Macy Conference on Genetics

Edited by
ROBERT S. KROOTH, M. D.

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PREFACE

Substantial progress appears to have been made during the last five years in the genetic study of cultured mammalian cells. The reasons for the increased activity in this field seem, at least to me, to fall into two categories.

First, on the technical level a great many events have made mammalian cell culture an easier and more repeatable experimental technique than it formerly was. I list the following (in arbitrary order) as among the most important:

1. The availability of antibiotics for use in cell culture media.
2. The availability of reliably pure biochemicals.
3. The clarification of the nutritional requirements of cultured mammalian cells.
4. The development of techniques that virtually always work for the initiation of human and other mammalian cell strains.
5. The development of techniques for an adequate visualization of the chromosomes of cultured mammalian cells.

Second, on the intellectual level, the striking advances that have been made in microbial genetics since World War II have led almost everyone to wonder whether analogous phenomena may also occur in cultures of mammalian cells.

Much of the progress that has been made is reported in this Fourth Macy Conference on Genetics. Although I have called this book *Somatic Cell Genetics*, most of it is devoted to but one aspect of the subject: Studies on cultured mammalian cells. Chapter I explores mammalian chromosomal genetics—this field was made possible by the fifth technical event I referred to above. The work here deals mainly with studies designed to show an association between the karyotype of a donor, or of a cell, and his or its phenotype. Chapter II deals with the phenotypic markers that have been used in cell culture to distinguish genetically disparate cells. In the second part of this chapter, experiments analogous to some of those performed in microbial genetics are reported.

The Preface published with the previous Macy Conferences on Genetics (and written by Dr. W. J. Schull) is also included here, following this one. It describes the history, purpose, and some of the editorial problems of these conferences. My own interpretation of the editor's role is that he is supposed to be only a collator. I have, therefore, left the documentation of statements, the citation of appropriate literature, etc., entirely to the individual participants. I have also permitted some revisions. It is difficult to enforce on one's colleagues restrictions that one would not wish to impose on oneself. In the main, however, this book is an accurate and nearly verbatim account of the conference.

ROBERT S. KROOTH

THE MACY CONFERENCES ON GENETICS

Somewhat over twenty years ago, the Josiah Macy, Jr. Foundation initiated a series of regularly scheduled conferences directed toward challenging problems in medicine and health. With time, firm but not immutable ground rules have been evolved for the conduct of these sessions. Thus, each conference group is to meet annually for a period of five or more years. Twenty-five persons, selected to represent a multi-discipline approach, are to participate in a meeting. These individuals are termed conference members if they have been selected to participate in a single meeting.

The purpose of each conference is the promotion of communication, the exchange of ideas. To this end, an informal give-and-take among the participants, members and guests, is encouraged. Structure and continuity are given the discussion by a leader whose function is to present some of the more interesting aspects of the problem under discussion. The participants are enjoined to interrupt this presentation with questions, criticisms, and comment. At their best, the interruptions lay bare the birth and maturation of an idea, and form, therefore, an essential part of the lessons to be gained from the conference process. To share these lessons as widely as possible, an edited transcript of the meeting is published. These transactions, which attempt to retain the spontaneity of the discussion, have aroused considerable interest and criticism. Comments range from an enthusiasm for, to a total rejection of, the personalized approach. Criticism, in the words of Frank Fremont-Smith, for many years the guardian of these conferences, "has been directed primarily to editorial permissiveness which has allowed in the final text, in some instances, too many questions, remarks, or comments which, although perhaps useful during a heated discussion, seem out of context and interrupt the sequence of thought." Clearly, not all critics recognize the narrowness of the path between spontaneity, on the one hand, and editorial permissiveness, on the other, nor the challenges which confront the editor.

WILLIAM J. SCHULL

PARTICIPANTS

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October 15–17, 1962

Princeton, New Jersey

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Urbana, Illinois

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New York, New York

*Dr. Charles W. Cotterman
Department of Medical Genetics
University of Wisconsin
Medical School
Madison, Wisconsin

Dr. James F. Crow
Department of Medical Genetics
University of Wisconsin
Medical School
Madison, Wisconsin

*Dr. H. Bentley Glass
Department of Biology
Johns Hopkins University
Baltimore, Maryland

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Stanford University
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Medical School
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Cleveland, Ohio

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Department of Zoology
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Berkeley, California

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California Institute of Technology
Pasadena, California

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The Wistar Institute
Philadelphia, Pennsylvania

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Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee

Dr. Robert DeMars
Department of Medical Genetics
University of Wisconsin
Medical School
Madison, Wisconsin

Dr. Harry S. Eagle
Department of Cell Biology
Albert Einstein College of Medicine
New York, New York

Prof. Boris Ephrussi
Department of Developmental Biology
Western Reserve University
Cleveland, Ohio

Dr. Stanley M. Gartler
Depts. of Medicine and Genetics
University of Washington
School of Medicine
Seattle, Washington

Dr. Leonard A. Herzenberg
Department of Genetics
Stanford University
School of Medicine
Palo Alto, California

Dr. Hilary Koprowski
The Wistar Institute
Philadelphia, Pennsylvania

Dr. Robert S. Krooth
Department of Human Genetics
University of Michigan
Medical School
Ann Arbor, Michigan

Dr. Jerome Lejeune
Institut de Progenèse
Faculté de Médecine
Paris, France

Dr. Klaus Patau
Department of Medical Genetics
University of Wisconsin
Medical School
Madison, Wisconsin

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Department of Genetics
University of Glasgow
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Dr. Waclaw Szybalski
McArdle Laboratory
University of Wisconsin
Madison, Wisconsin

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CHAPTER I: HUMAN CHROMOSOMAL GENETICS

The Study of Gross Chromosomal Abnormalities by Dr. Jerome Lejeune

Neel: Dr. Jerome Lejeune is going to open the discussion for this Fourth Macy Conference. I should warn you, Dr. Lejeune, that you should not assume an extensive background on the part of your audience and, if you attempt to, you may find yourself summarily halted.

Lejeune: The main difficulty with human chromosomes is that, first, before looking at any abnormality, we have to be sure that we can recognize the chromosomes themselves. It may seem a little childish here to come back to the human karyotype in its normal form, but I just suppose it is necessary that we first recognize what is normal before saying something is abnormal.

Figure 1 shows the 47 chromosomes of a mongol boy, and Figure 2 shows the karyotype, according to the Denver classification.

The main problem is not so much to distinguish the big and the small chromosome, because, for example, nobody would have difficulty in finding the big acrocentrics and the small acrocentrics and so on, but the difficulty is whether we are really entiled to give a number, as set forth in the Denver agreement, to every one of the medium-sized chromosomes.

The problem has been mainly surreptitiously discussed, and I suppose that Dr. Patau will talk about it in a few moments. We wanted to know whether, really, the medium-sized chromosomes were different pairs, or if some of them were in the tetraploid state in our species. What we did was to plot the length of each element and the position of its centromere on 50 male cells and on 50 female cells. The centromere index was calculated as the ratio between the short arm and the total length of the element; that is, the distance, expressed as percent of the total length of the chromosome, of the centromere from that end of the chromosome which is closest to the centromere.

Figure 3 shows the results. In this figure there are 1550 points,

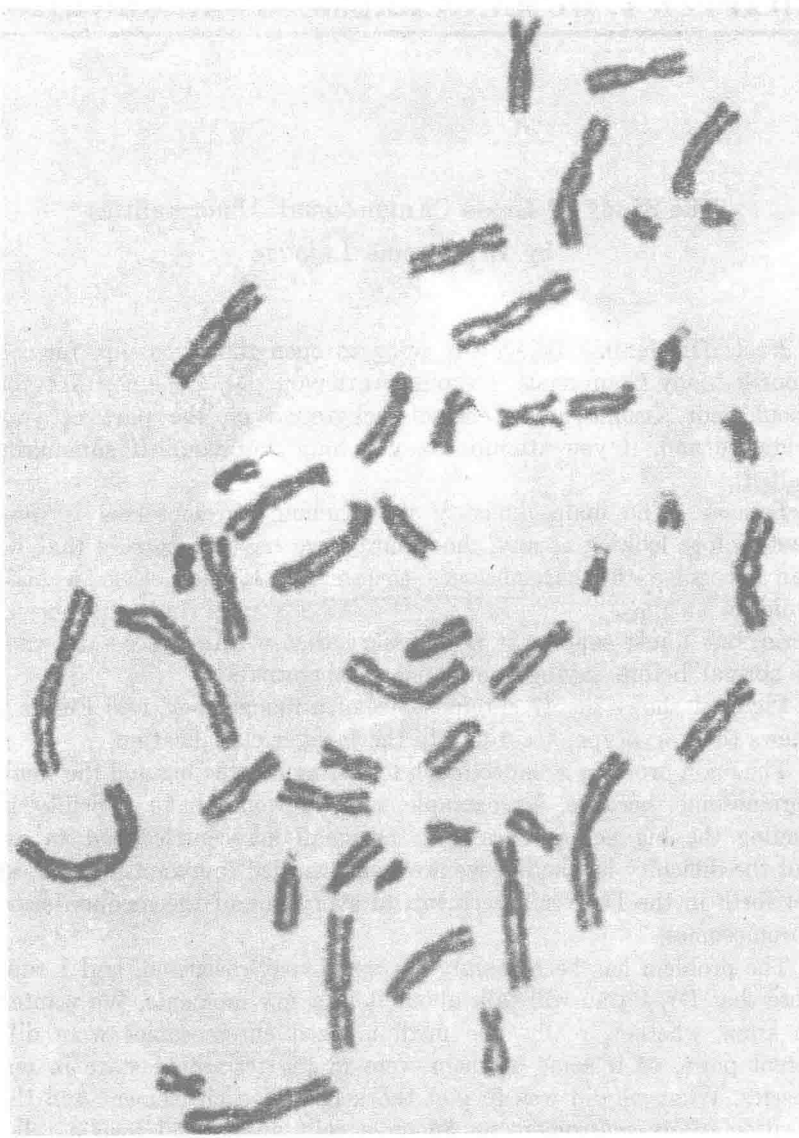

Figure 1. The 47 chromosomes of a Mongol boy.

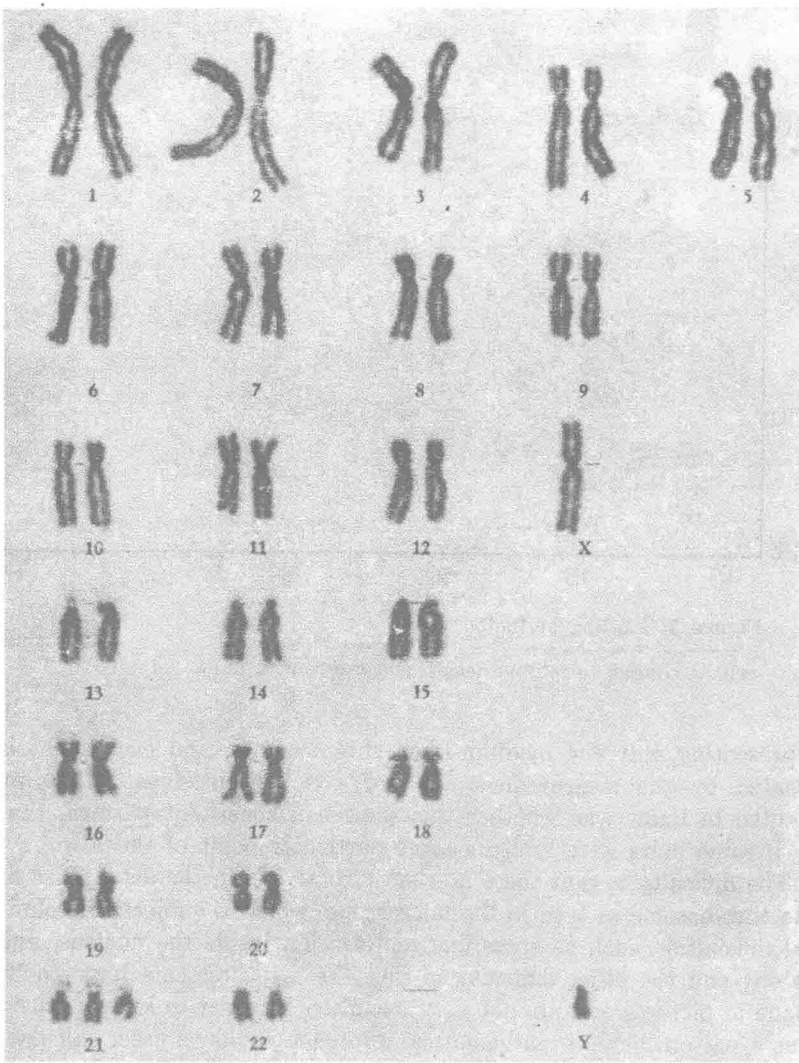

Figure 2. The karyotype of a Mongol boy. The trisomy is typical, with three satellited chromosomes. Faint satellites are seen in one of the 22 chromosomes.

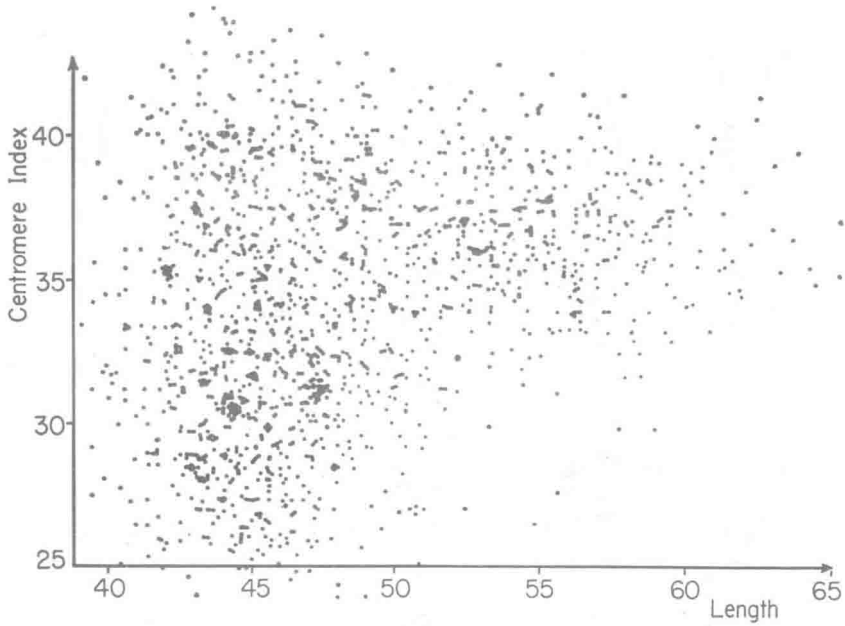

Figure 3. Plotting of the medium-sized pairs of 50 male and 50 female cells, according to relative length and centromere index.

representing only the medium-sized chromosomes, and each point is located by the measurements on only one chromosome. What we wanted to know was whether this showed a consistent pattern, that is, if some pairs were lying in some particular point of the plane.

The difficulty is that there are two errors: one in the actual size of the chromosome as seen in the microscope, which is subject to technical difficulties, such as spreading and tension inside the nucleus, and so on; and the other difficulty is that the measurements have to be made in pictures and are not very accurate. In order to know if there was a pattern here, a mathematical approach could be used, calculating any local concentration for any given area of the plane. But that is very cumbersome, and I could not do that, so what we did was just an optical trick.

Figure 4 will show us the result, which was to outfocus the projection so that instead of one point, we get a big circle for each point. I am sorry this does not show so well in the figure. There are points of concentration, which show much better when the light is not so bright.

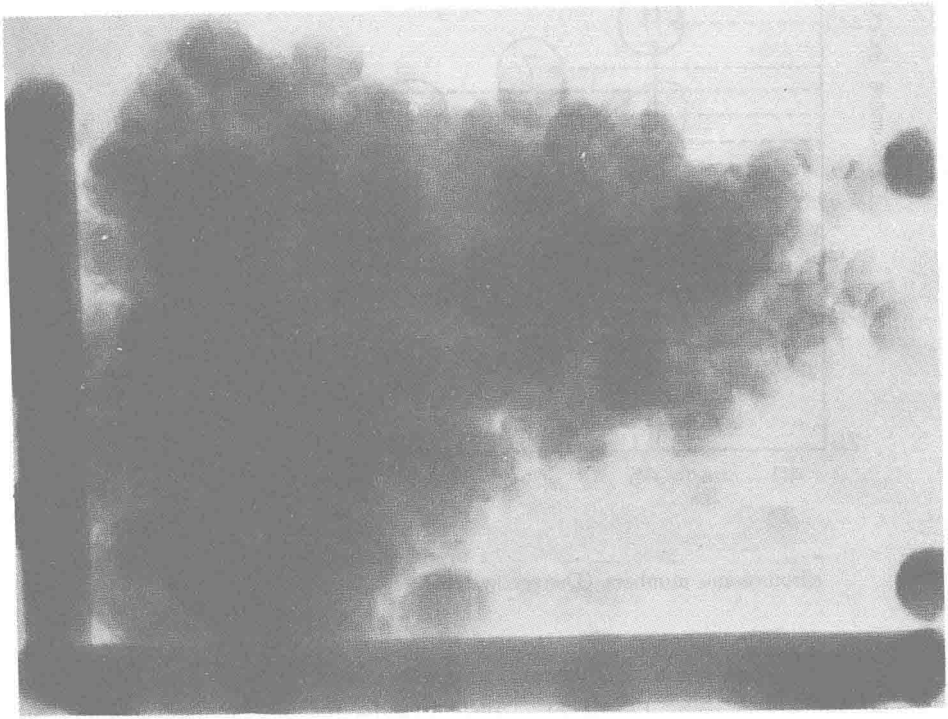

Figure 4. Out-of-focus picture of the plot given in Figure 3.

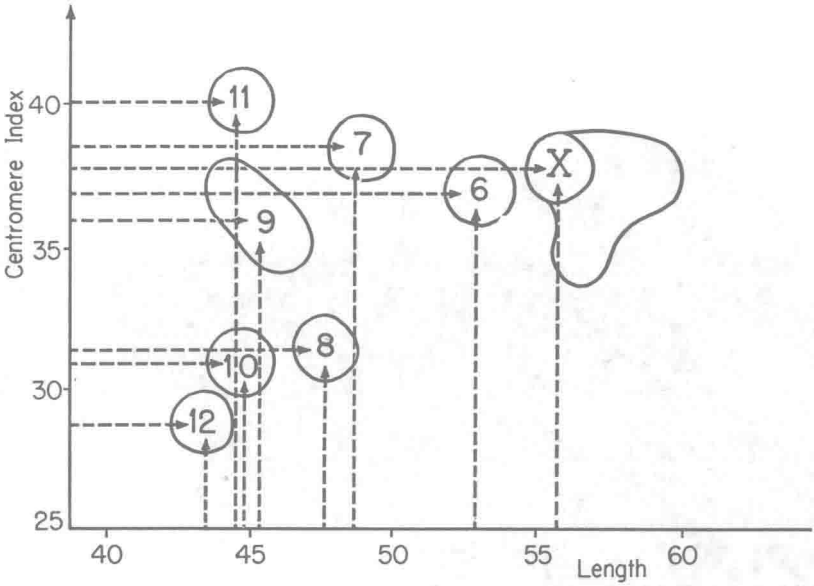

Figure 5. Diagram of the points of concentration and the corresponding chromosome numbers (Denver system).

Figure 5 shows us a diagram which is drawn from Figure 4, and shows where these points are. According to the Denver agreement, we have to put the chromosomes in order, beginning with the biggest one, and going on to the smallest one. The abscissa indicates the length of the chromosome, and the centromere index is indicated on the ordinate.

The problem was, afterward, to detect where the X chromosome was, and, to do this, we had to use a trick which probably is not perfectly justified, but we could not find another way which was better. The trick was to have a mean length for every cell; that is, for the medium-sized chromosome, we added the lengths to get the sum, which is the length of 15 chromosomes and of 16 chromosomes contained respectively in each male and female cell. We have a mean length for 50 male cells, and we have a mean length for 50 female cells. Now, if we assume that the difference between the two means is due to the presence of one X in the male and two X's in the female, it comes out that the difference is 0.56. For the centromere position, we did the same calculation; that is, the centromere index being, for

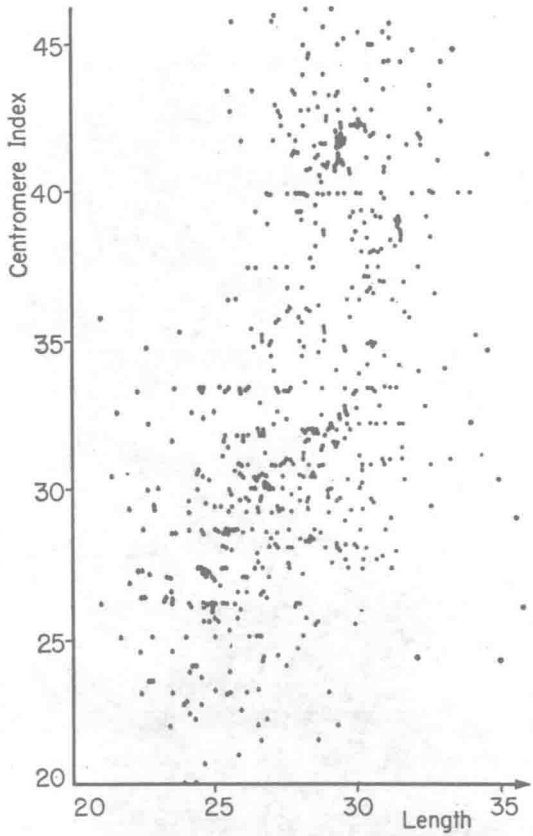

Figure 6. Scatter diagram of measurements on chromosomes in the 16 to 18 group. See Figure 3 for complete legend.

example, 0.33 in a given chromosome, we add up all the chromosomes of one cell, and then we get a mean sum for all the 50 female cells; in this way we get a total of centromere indices for each of 50 female cells and for each of 50 male cells.

If we consider that the centromere is a kind of weight, the difference is related to the position of the centromere of the X, and it turns out that the difference is 0.38.

Returning to Figure 5, we can locate the position corresponding to (38, 56), shown by dotted lines and arrows. This position is an area

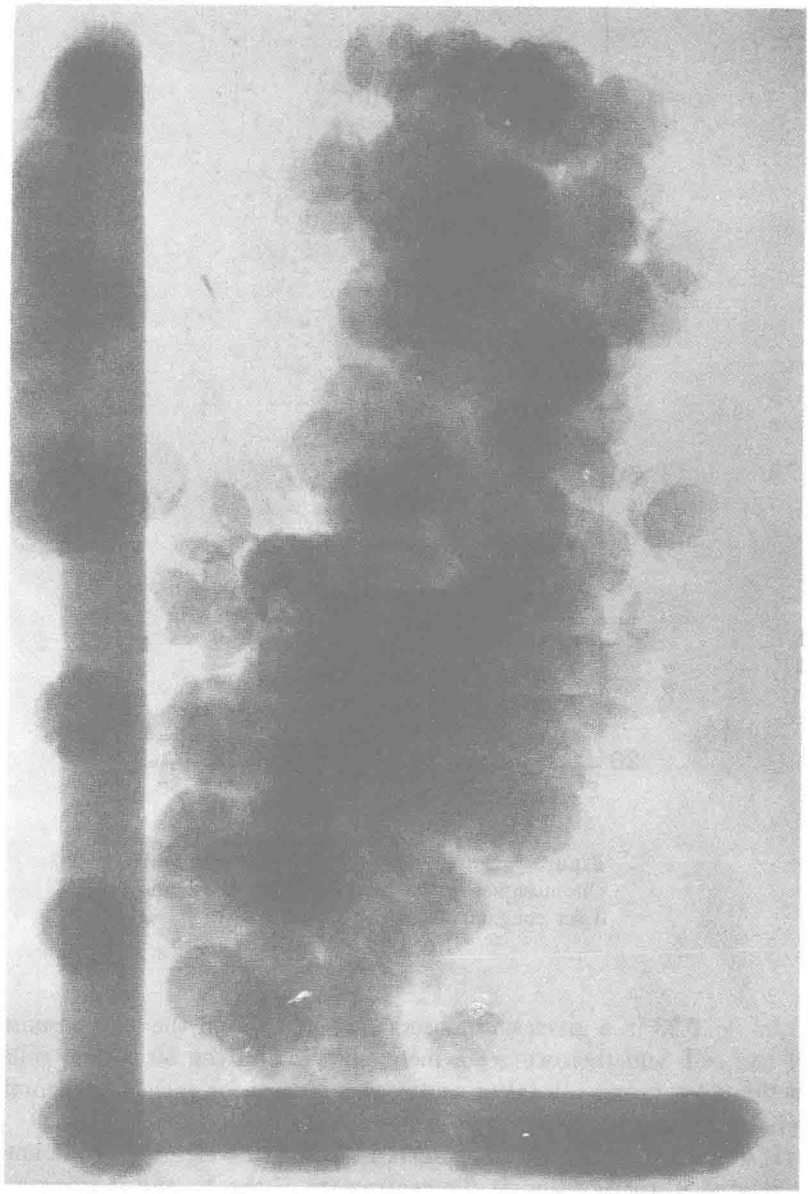

Figure 7. Out-of-focus picture of the plot given in Figure 6.