

MAMMALIAN CHROMOSOMES IN VITRO

I. The Karyotype of Man

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THE following paragraph, quoted from Fischer¹, well states some of the advantages of studying chromosomes in tissue culture:

Cultures present extremely favourable conditions for an investigation of the cell division. Entirely apart from the fact that the tissue culture offers a good general view of the individual phases of the division, it has the great advantage over histological section preparation that nothing is lost, something that may well happen in the case of the latter when first the microtome knife has cut the tissue. Another advantage of the tissue culture is that the growth zone often is but a single layer in thickness and therefore presents the best conditions for direct observation, photography or cinematographic recording.

Besides these the fixation of tissue cultures is direct and instant. In section or smear preparations, no matter how skillful the operation, a minute or two may be lost during dissection thus causing some undesirable effects, such as clumping of chromosomes. The fixatives are more effective because their penetrating power is enhanced due to the thin sheets of outgrowth in tissue culture.

One of the earliest workers on human chromosomes *in vitro*, Kemp^{3,4}, used tissue cultures of embryonic heart, liver and spleen and made some important inroads with this technique. Unfortunately, this approach has been almost completely neglected by cytologists.

Materials and Methods

Cultures of embryonic skin and spleen were made from a four month old male fetus. The tissues were cut into square pieces, approximately 2 mm. on each side for the skin and

1 mm. on each side for the spleen. Each explant was mounted on a $\frac{1}{8}$ " square cover slip in a clot composed of equal parts of extract from seven-day chick embryos and heparinized cockerel plasma and sealed with paraffin on a depression slide. The skin cultures received approximately 0.05 ml. of malignant human ascitic fluid as a liquid nutrient but nothing was added to the clots of the spleen cultures. After five days of incubation at 37°C., the skin cultures were fixed in pure methyl alcohol and the spleen cultures in Helly Zenker's. The skin cultures were stained in a combination stain consisting of May-Greenwald and Giemsa, known as Jacobson's method, while the spleen cultures were stained with hematoxylin-eosin-azure.

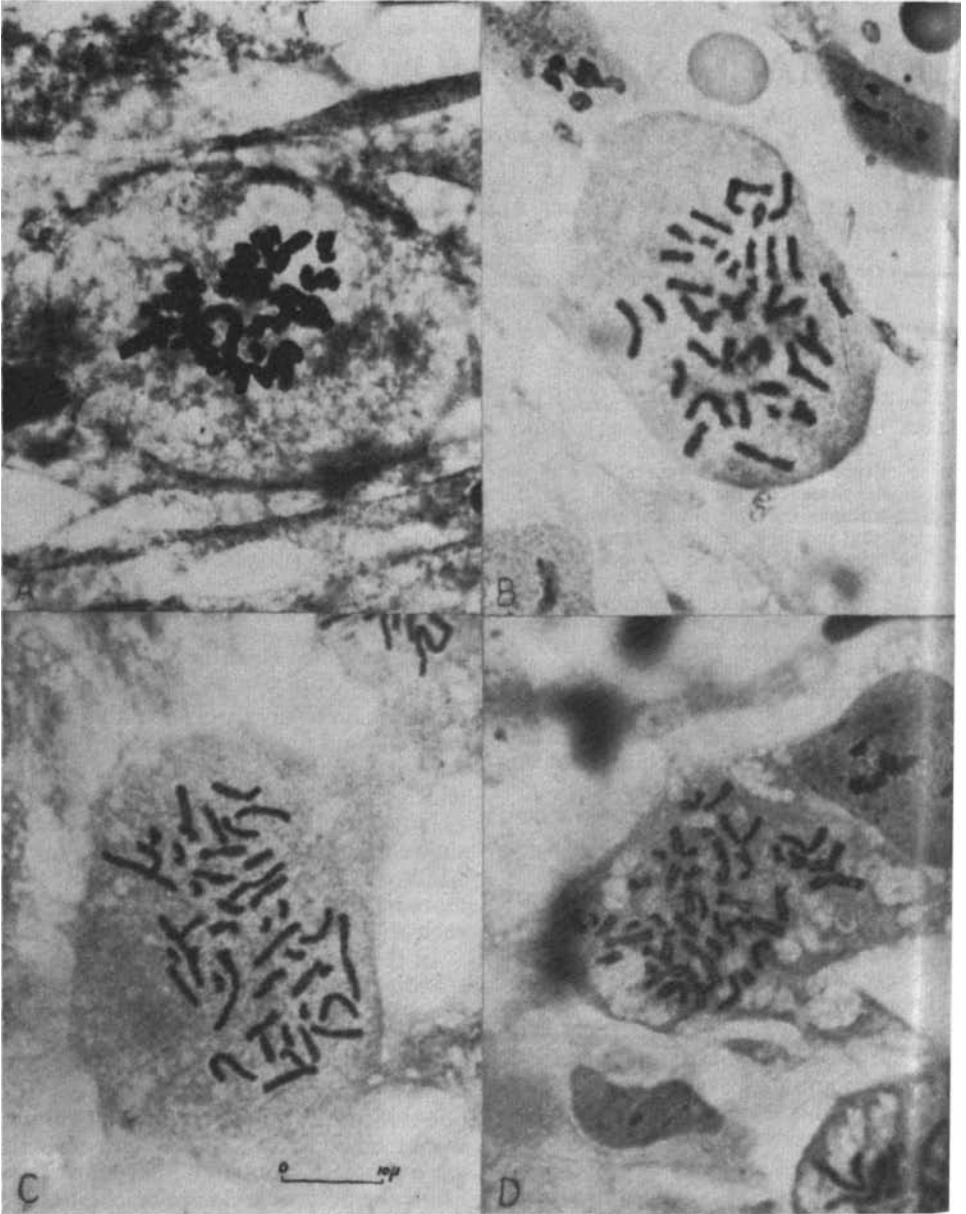
Results

The elements in the zone of outgrowth of the skin cultures were mainly epidermal cells and fibroblasts. Unlike the epidermal cells in cultures of adult skin in which there is very little mitotic activity (Hsu²), embryonic epidermal cells showed some regular mitotic figures, while fibroblasts exhibited considerably more mitotic activity. Figure 11A shows such a fibroblast at metaphase.

In these cells various degrees of overspiralization of chromosomes were observed and many cells contained small, compact chromosomes which made the karyotypic study difficult. Anaphasic movement was remarkably normal, with no evidence of abnormalities. Counts were made only in four cells in which all were shown to contain 48 chromosomes.

For the purpose of karyological studies, the spleen cultures were more desirable because their chromosomes at prometaphase were very well outlined and much better dispersed. Figures 11B to 12D

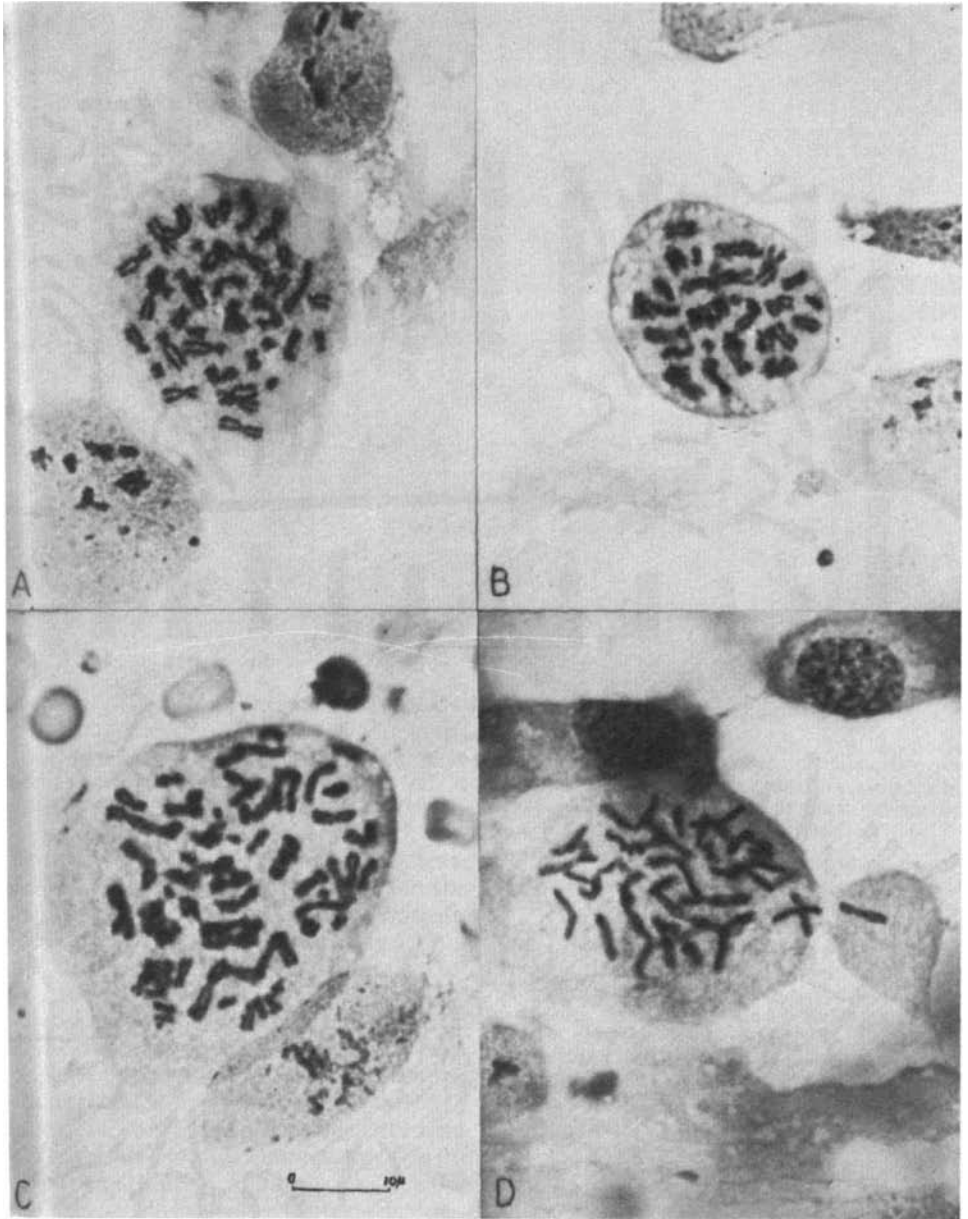
*Damon Runyon fellow: aided by a grant from the American Cancer Society (CP-12D) administered by C. M. Pomerat. Grateful acknowledgement is made to Mmes. Mildred Finerty, Helen Dunton and Patricia Johnson for indispensable assistance with the preparation and staining of the cultures. I am also indebted to Mr. George Lefeber for his help in preparation of the photographs.



HUMAN CHROMOSOMES

Figure 11

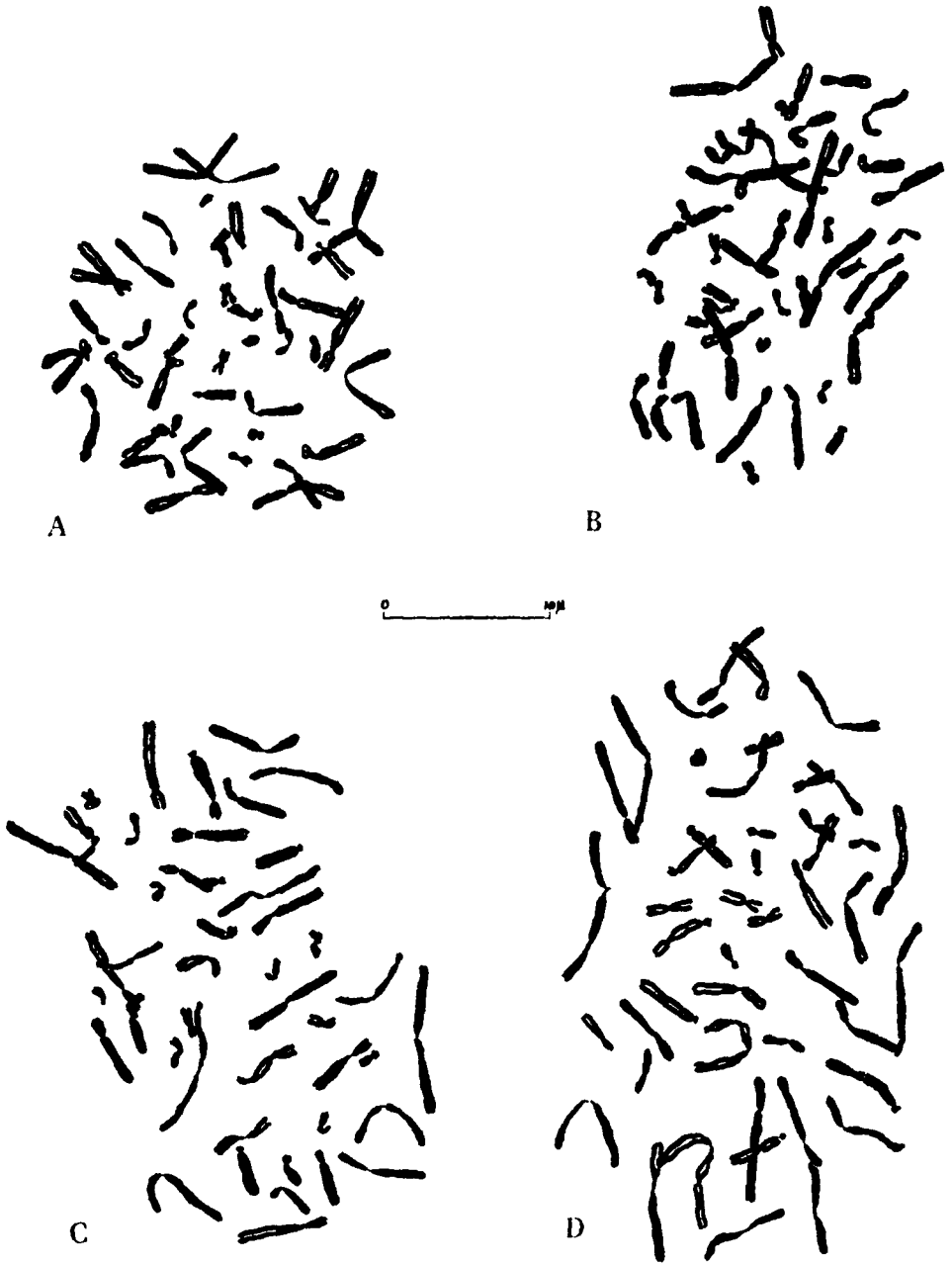
Figures 11 to 13 present cells from a four month human fetus cultivated *in vitro* for five days. *A*—A cell from a skin explant, presumably of fibroblastic type, showing marked overcondensation of chromosomes. Fixed in methyl alcohol stained according to Jacobson's method. *B-D*—Cells from splenic explants believed to be fibroblasts. Fixed in Helly Zenker's followed by staining with hematoxylin-eosin-azure.



EMBRYONIC SPLEEN CELLS

Figure 12

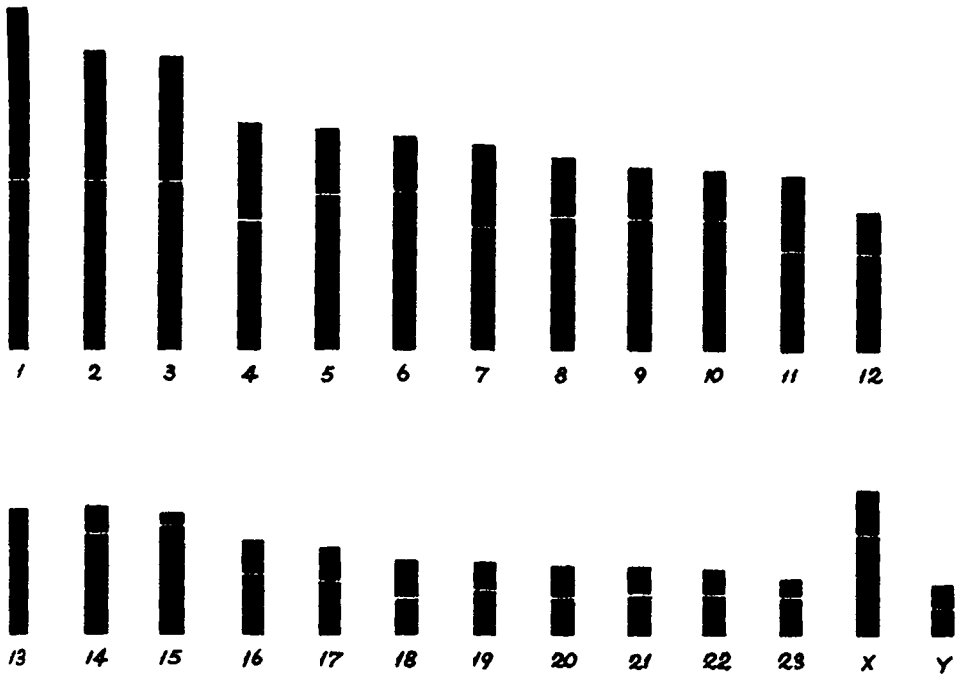
Cells from splenic explants. From the same source as the tissue illustrated in Figure 11.



CHROMOSOME CONFIGURATIONS

Figure 13

Camera lucida drawings of cells of the type represented in Figures 11 and 12.



THE HUMAN CHROMOSOMES

Figure 14

Diagrammatic representation of the haploid set of human chromosomes including the sex pair as seen in preparations of human embryonic spleen cultivated for five days *in vitro*. The autosomes are numbered according to their length, regardless of the position of the centromeres.

represent selected examples of splenic elements. In these cells not only can the numbers of the chromosomes be critically counted, but also their relative length and the position of the centromeres can be studied in detail.

In her work on bone marrow, La Cour⁵ reports that there is a difference in the charge of nucleic acid between promyelocytes and proerythroblasts. In promyelocytes the chromosomes are long and thin, relatively undercharged with DNA and hollow spindles are usually formed at metaphase. In proerythroblasts the chromosomes are over-spiralized, heavily charged with DNA and the metaphase plates are mostly multipolar. In the spleen cultures, although most cells in division stages probably were fibroblasts, many exhibited long and thin chromosomes and some behaved as if they were in C-mitosis. A few metaphases also showed hollow spindles.

Figure 13A is a typical example of the C-mitotic cell, in which all the chromosomes had their chromatids widely split except the centromeres. Figure 13B represents an early anaphase in which the centromeres are also separate but there is no sign of moving to the poles. Apparently such cells form tetraploids.

Counts were made of 124 cells from the spleen cultures among which 91 or approximately 73.4 percent were shown to contain 48 chromosomes. One cell was probably a hypotetraploid, showing 91 chromosomes (Figure 13C). The rest of the cells had chromosome numbers as follows: four cells with 49 chromosomes, 11 cells with 47, 11 cells with 46, five cells with 45 and one cell with 44. Obviously polyploid cells were not common in the tissue before it was cultured.

More than 70 anaphases and early telophases were examined but no visible abnormalities, such as lagging, bridge formation, uni- or multipolarization, were noticed. No micronuclei were observed in either the skin or the spleen cultures. The most probable cause of the deviation of the diploid chromosome number would thus be non-disjunction, but it is extremely difficult to obtain accurate direct counts from anaphases. Another possible way

of eliminating a chromosome from a set is shown in Figure 12D, where a single chromosome is cut out from the main cell at metaphase. This phenomenon was encountered twice in the series. Presumably the fraction of cytoplasm containing one or two chromosomes cannot survive.

The morphology of the chromosomes has been studied to determine the karyotype of man.

A diagrammatic representation of the haploid set of human chromosomes, including the sex pair, is shown in Figure 14. The autosomes are numbered according to their length regardless of the position of the centromeres. There are no true telocentric chromosomes, every chromosome has two definitive arms. The lengths of the chromosomes when lined up form a graded series without a sharp break between long and short members. The longest is a pair of V-shaped autosomes with the two arms nearly equal in length. Most chromosomes are J-shaped, and pair number 15 has the shortest second arm, almost in the form of a knob. The only way to determine the sex pair is by matching the chromosomes in pairs. In the matching of chromosomes in human spermatogonial mitosis, Painter's⁶ result showed that the *X* is a medium-sized chromo-

some, but in La Cour's⁵ drawing the *X* appears to be a rather long *J*. In our material two unmatched chromosomes, one a medium-sized *J* and the other the smallest chromosome, are presumably the *X* and the *Y* respectively.

Addendum. It was found after this article had been sent to press that the well-spread metaphases and the seemingly C-mitotic anaphases were the results of an accident. Instead of being washed in isotonic saline, the cultures had been washed in hypotonic Tyrode solution before fixation. Furthermore, it was found that Dr. Arthur Hughes of the Strangeways Research Laboratory, Cambridge, England, had been carrying on experiments on the effect of hypotonicity upon dividing cells and his findings were almost identical with ours. We owe our sincere thanks to Dr. Hughes for allowing us to read his original manuscript prior to its publication.

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AMERICAN TOMATO YEARBOOK

THE 1952 edition of the *AMERICAN TOMATO YEARBOOK* has just come off the press. It is again edited by Dr. John W. Carncross, Rutgers University College of Agriculture, and contains much information for the tomato grower, the tomato dealer and shipper, the tomato canner, and the tomato research specialist.

The main function of the Tomato Genetics Cooperative at present is to compile and to distribute to members a Report issued annually that embraces the following parts:

Part I. Research Notes. This part, consisting of short articles relating to tomato genetics is probably the most useful section of the report. Part II. Directory of Members. Part III. List of Available or Desired Stocks. Part IV. Bibliography of papers on tomato genetics and breeding published during the preceding year. Part V. Financial Statement.

The book is profusely illustrated with up-to-date charts and graphs giving a complete picture of the tomato industry. In addition there is much statistical information of vital importance. This includes a tabulation of states giving current data on tomato yield, acreage and production for both processing and fresh market, statistics on tomato juice, tomato pulp, tomato catsup, tomato imports and exports.

The requirements for membership in the Tomato Genetics Cooperative are an active interest in the work and willingness to pay the annual assessment. Anyone interested in joining or in securing additional information should communicate with Dr. John W. Carncross, Rutgers University, New Brunswick, N. J. Comment on any aspect of the activities of the Tomato Genetics Cooperative is always welcome.