AN ANALYSIS OF THE HUMAN CHROMOSOMES IN THE LEUCOCYTES OF THE CIRCULATING BLOOD

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One of the most important achievements of cytogenetics in recent years has been the discovery that human chromosomes may be examined in the leucocytes of the circulating blood [6, 9, 14, 15]. This technique has led to the discovery of a whole number of new chromosomal diseases, and great possibilities have been opened up for studies in radiobiology and cosmic medicine.

Here we report a method of analysis of the human chromosomes in blood, as developed at the laboratory of radiation genetics, Institute of Biophysics, AN SSSR, under the direction of Corresponding Member AN SSSR N. P. Dubinin.

I. CULTURE OF THE LEUCOCYTES

Collection of Blood. Separation of the Leucocytes.

1. A sterile syringe moistened with heparin (10 mg/powdered heparin/ml) is used to withdraw 10 ml of blood from a vein in the elbow.

2. To this amount of blood 1 ml of 10% solution of gelatin is added (ultimate concentration of gelatin in the blood should be 1%). The test tube containing the blood is shaken, and placed in a holder.

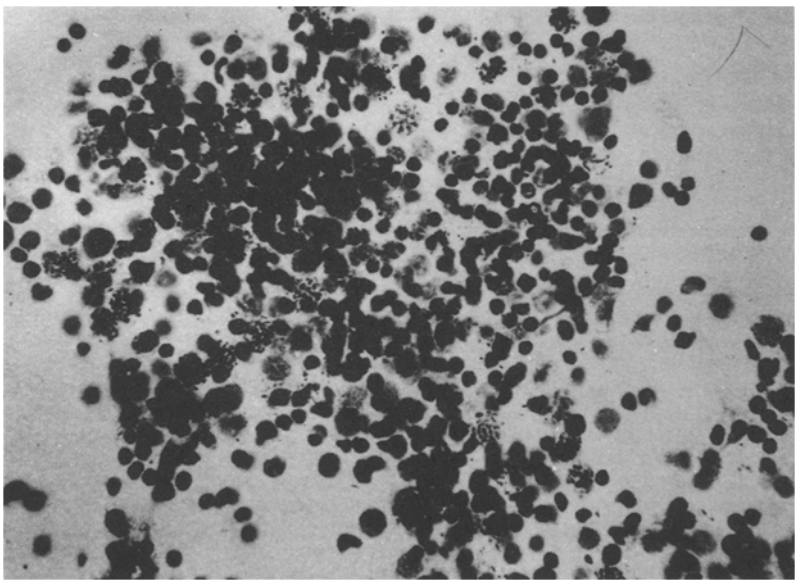
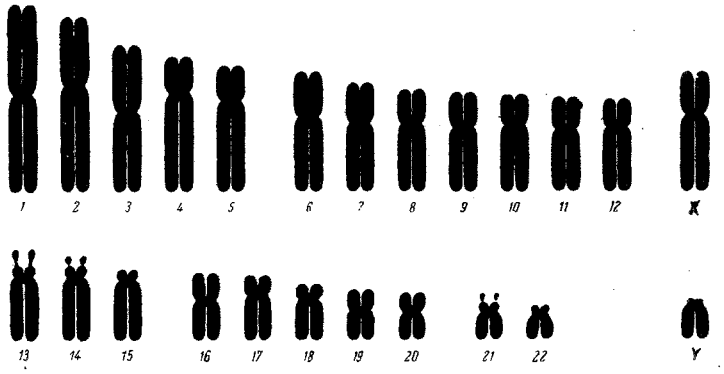


Fig. 1. Mitoses of leucocytes in human circulating blood 72 h after culture with phytohemagglutinin.



	1	2	3	4	5	6	7	8	9	10	11	12	X
Short limb	41·5	31·6	30·8	16·6	16·0	20·6	18·6	15·3	16·2	13·8	15·0	12·4	19·9
Long limb	44·7	49·1	36·9	45·7	42·2	34·9	31·5	31·2	29·2	30·7	28·5	29·9	35·2

	13	14	15	16	17	18	19	20	21	22			Y
Short limb	4·8	5·2	4·8	12·1	9·0	6·4	9·6	9·3	4·2	3·9			2·0
Long limb	28·9	28·8	27·1	18·4	20·5	19·2	13·9	12·2	12·4	11·6			15·7

Fig. 2. Idiogram of human somatic chromosomes.

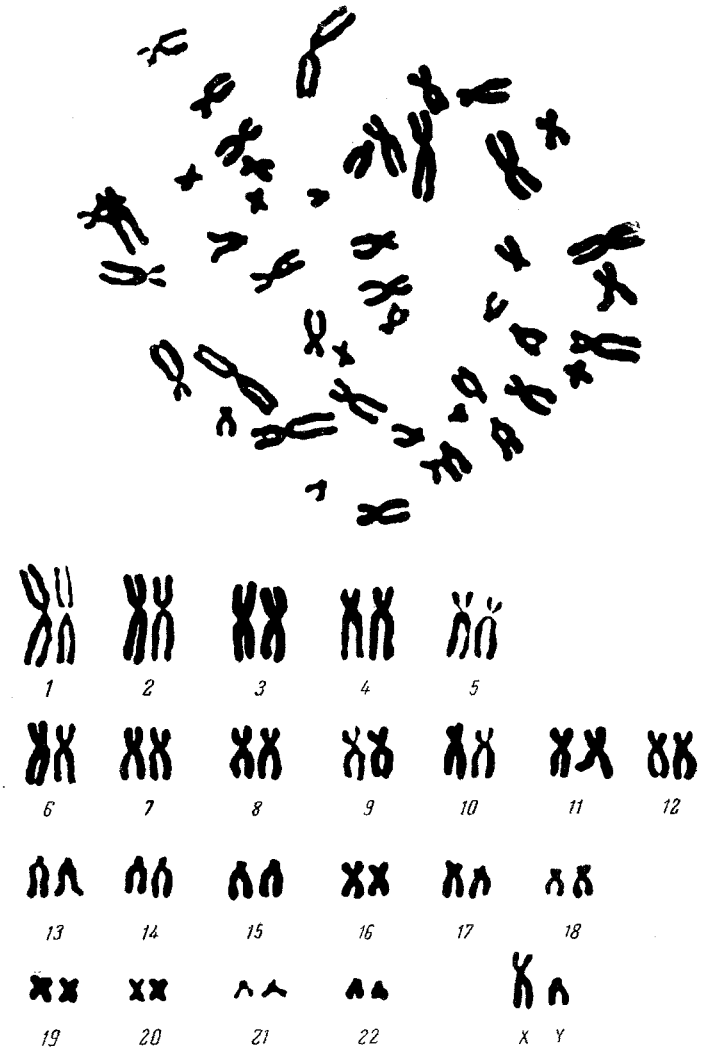


Fig. 3. Set of human male chromosomes. Metaphase plate, and caryogram.

3. After 15 min the erythrocytes begin to settle, and the buffy coat which settles upon them and which contains the leucocytes is sucked off into a new tube. In this way 60% of all the leucocytes contained in the blood may be removed.

The leucocytes may be removed by centrifugation at 350 revs/min for 15 min. However, because of the small difference between the specific weight of the formed elements of the blood (erythrocytes 1.092, leucocytes 1.065, thrombocytes 1.032, and plasma 1.026) the yield of leucocytes in only 40%.

Some authors [3] recommend separation of the lymphocytes for culture by absorption on glass walls; the method is to filter the heparinized blood at a small positive pressure.

The method we have worked out for obtaining the suspension of leucocytes by means of gelatin is the most rapid, and is more convenient than the methods proposed previously. The advantage is that during the process of separation the leucocytes are not subjected to the mechanical disturbance of centrifugation, filtration, or cooling; also the gelatin is a useful addition to the culture medium, and does not prevent leucocytes undergoing division.

Explanting the Leucocytes

4. In a Goryaev chamber a count is made of the leucocytes contained in the separated plasma. This volume is then diluted with medium No. 199 (containing 50 units of penicillin per ml) until the ultimate concentration of leucocytes is 1.5 million-2 million cells per mm^3 of suspension (15-20% plasma and 85-80% medium No. 199).

5. Next 1.5-2 ml of the suspension of leucocytes is poured out into sterile penicillin flasks for culture. The residual air from the lungs, containing up to 5% CO_2 was blown through the whole fluid.

6. To each flask is added 2-3 drops of a solution of phytohemagglutinin*. The flasks are tightly closed with rubber corks, and placed in a thermostat at 37°.

Culture of the Leucocytes. Colchicine-hypotonic Treatment. Fixation of the Culture.

7. The flasks with the culture are kept in the thermostat for 3 days. During this time a gaseous mixture containing 5% CO_2 [15] should be passed through the flasks, although it is not essential to do so.

8. Next, 65-70 h after the culture had been started, to each penicillin flask 0.5 μg/ml colchicine is added. The culture is gently stirred, and placed in a thermostat at 37° for 3 h. Then the contents of the flask are once more poured out into tubes, and centrifuged for 10 min at 800 revolutions per min.

9. The supernatant fluid is decanted, and the settled leucocytes are resuspended in 5 ml of hypotonic solution (1 part of Hanks solution, 3 parts distilled water) at 37-40°. The suspension of leucocytes is once more placed in the thermostat at 37° for 30 min.

10. After incubation the hypotonic solution is removed by repeated centrifugation, and to the residue on the walls of the test tube 5 ml of fixative cooled to 4° is added; the fixative consists of 1 part glacial acetic acid and 3 parts of methyl alcohol. Fixation is continued in the cold for 45 min at 4°. The leucocytes are resuspended after the first 15 min.

11. The fixative is removed by centrifugation, and the residue of leucocytes is carefully mixed with 0.5 ml of fresh fixative.

II. STAINING AND MOUNTING THE PREPARATIONS

12. Object glasses free from grease are cooled with solid carbon dioxide, and then a small amount of the suspension of leucocytes is vigorously scattered on them from a Pasteur pipette. The preparations are dried over a gas flame.

* Phytohemagglutinin is the hemagglutinating fraction of the protein of the kidney bean; it is able to induce mitoses in human leucocytes in blood cultures. It is separated from the extracts of the beans by a 2-stage salting-out with ammonium sulphate, removal of the ballast proteins, and by selective precipitation of the fractions of the phytohemagglutinins in aqueous solution at pH 5.8 [5, 10-13].

As our experiments have shown, phytohemagglutinin may be separated from the Moscow white, green pod (B-556)" and "pearl" varieties of Soviet kidney beans.

13. The preparations are stained with a 1% solution of acetic acid and orcein for 30 min. Then the stained preparation is treated with a 10% solution of acetic acid for 10 sec, with acetone (1) for 1 min, with acetone (2) for 3 min, with carbol-xylol for 1 min, with xylol (1) for 1 min and xylol (2) for 5 min, and then fixed in Canada balsam.

III. ANALYSIS OF THE CHROMOSOMES

Examination of the Preparation. Working Out the Caryotype. Determination of the Modal Number of Chromosomes.

Under the low power a large number of leucocytes can be seen, and of them 15-20% are undergoing mitosis (the mitotic index of the culture depends upon the activity of the phytohemagglutinin) (Fig. 1).

The analysis of the chromosomes was made with an immersion objective. For this purpose we selected metaphase plates where the chromosomes were scattered, and not lying over each other. The metaphases were drawn, and from the drawings a count was made of the chromosomes; in man, in the somatic cells the number is 46 [1, 4, 16], comprising 44 autosomes and 2 sex chromosomes—XX in women and XY in man.

The chromosomes are set out systematically in the idiogram of Fig. 2 where they are arranged in the order of decreasing length, and are identified from the position of the centromere, the presence of satellites, and from the chromosomal indexes* [2, 8].

To establish the caryotype, the chromosomes are photographed, cut out, and laid out as an idiogram (Fig. 3).

The modal number of human chromosomes was calculated from an examination of 100 metaphase plates, and the percentage of cells with an abnormal number of chromosomes was calculated. Usually it does not exceed 3-7% [7].

The leucocytic method is the most convenient means of studying human chromosomes. It enables structural anomalies associated with hereditary diseases and radiation sickness to be revealed, and for the course of the chromosomal changes taking place during the pathological process to be revealed.

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*The chromosomal indexes are made in terms of 3 measurements: 1) the relative length of each chromosome to the length of the chromosomes of the whole caryotype, 2) the relative length of the limbs of the chromosomes, 3) the ratio of the length of the short limb to the length of the whole chromosome.