Patients with congenital anomalies and leukemia, visiting the Nehru Hospital, Chandigarh, Rajindra Hospital, Patiala and various other clinics were studied for their chromosomal make up. Their clinical data, family history, past and present histories were recorded in a proforma. In addition, 25 normal cases belonging to various age groups were also studied for their chromosomes.

The chromosomes were analyzed mainly by the direct culture of aspirated bone-marrow, but sometimes the leucocytes were also cultured.
1. **DIRECT BONE MARROW CULTURE TECHNIQUE**

One ml. of the sternal or iliac crest bone-marrow was aspirated with a sterile syringe and mixed with 1 ml. of G.K.N. (Glucose potassium saline solution) sterile solution (Merchant et al., 1960) containing 2 mg. E.D.T.A. (Ethylene diamine tetraacetate) in a siliconized centrifuge tube. This was allowed to settle at 4°C. for 30 minutes. Marrow particles floating at the top were thoroughly mixed with the supernatant plasma by repeated pipetting with a sterile pasteur pipette to release the cells trapped in fat. This was then centrifuged at 500 r.p.m. for 5 minutes. The uppermost layer containing fat droplets was discarded and the supernatant plasma along with the buffy coat was transferred to another sterile siliconized centrifuging tube. Two ml. of sterile Hank's balanced salt solution containing 0.05% colchicine was added to the cell suspension and the mixture was incubated at 37°C. for 1 hour. The cells were then harvested by centrifuging at 500 r.p.m. for 5 minutes, washed once with G.K.N. solution and then subjected to hypotonic treatment with 3 ml. of previously heated sodium citrate solution for 10 to 15 minutes at 37°C. The cells were again sedimented by centrifugation at 800 r.p.m. for 5 minutes and the supernatant was
discarded. Initial fixation of the sedimented cells was then carried out slowly by drop wise addition of 3 ml. of freshly prepared methanol acetic acid (3:1) mixture for at least 30 minutes at 4°C. This was followed by 3 more changes of fresh fixative. Chromosome preparations were made by the air drying technique and stained with carbol fuchsin.

2. LEUCOCYTE CULTURE TECHNIQUE

In many cases attempts were made to culture the leucocytes following the technique of Mellmann (1965), the outline of which is given below:

a) 5 to 10 ml. of the venous blood was drawn aseptically in a 10 ml. syringe containing 0.1 ml. of the aqueous heparin.

b) Aspirated the blood from the needle and a small space below the hub of the syringe. Wiped the needle with alcohol and covered with a needle-cover. Made the syringe to stand at room temperature for about an hour, so that 30-40% of the blood volume became visibly cleared of the red cells.

c) Then the plasma was extruded in a sterilized culture vessel containing 1 ml. of autologous plasma, 6 ml.
of basic medium TC 199, 0.05 ml. of bactophytohaemagglutinin and 0.02 ml. of solution containing penicillin (100,000 units/ml.) and streptomycin (100 mg/ml.).

d) Incubated the vessel at 37°C. with an air tight cap.
e) On the third day of culture (60-70 hours), added 0.05 ml. of colchicine to give a final concentration of 0.04 mg/ml.
f) After 2-4 hours, suspended the cells in the medium and transferred them to a centrifuge tube, spun for 5 minutes at 500-800 r.p.m. removed the medium and added instead 5 ml. of Hank's balanced salt solution. Respun the material at 500 r.p.m. for 5 minutes, discarded the Hank's balanced salt solution, added about 2 ml. of distilled water, and allowed to stand for 8-10 minutes at 37°C.

g) Resuspended the cells and centrifuged, discarded the supernatant and fixed the cells in methanol acetic acid (3:1).
h) After making a change of fixative, the slides were prepared as given above in the bone-marrow culture technique.
CULTURING AND KARYOTYPING ALL CHROMOSOMES

In all the cases, counts were made at metaphase. Only good chromosome plates were considered for this purpose. Unusually overspread or clumped chromosome plates were not considered at all. The counting was done with the help of a camera-lucida. Any bias in counting was avoided by dividing a chromosome plate into 4 regions in a camera-lucida image (Boddington et al., 1955) with pencil lines. The chromosomes in each region were counted thrice to give a consecutive number and then the different partial counts were added to give the full chromosome number in a plate.

Excellent plates were microphotographed. The karyotype was constructed by cutting the chromosomes from the microphotograph and arranging them according to the Patau's (1961) classification.

SEX-VIOMAT SUSPENS

The inside of the cheek was scraped gently but firmly with a metal spatula and the mucoid deposit was smeared thickly on the surface of a clean slide. The slide was immersed immediately in 1:1 ether - 95 per cent ethyl alcohol fixative for 2 hours. Following fixation, the smear was hydrated by passing through 70 % and 50 % ethyl alcohol after which it was washed in 3 changes of distilled water for 3 to 5 minutes each.
It was then stained with cresyl violet acetate in which it was allowed to stand for 30 minutes. Subsequently, it was passed through 2 changes of 95% ethyl alcohol for 2 minutes each, then rinsed twice in absolute alcohol and cleared in 2 changes of xylol for 5 minutes each. It was then mounted in DPX.
All the microphotographs given in the thesis were shot by using Carl Zeiss microscope and attachment with 3X eye piece and 100X objective and were enlarged 3 times. But some photographs were enlarged 5 times.