Material & Methods
During the period 1985-1988, 100 patients with multiple congenital abnormalities and cancers were chromosomally analysed. Blood samples of the patients were collected from different wards, such as Paediatric Surgery and Premature nursery of the Nehru Hospital attached to the Post-Graduate Institute of Medical Education and Research, Chandigarh.

Information regarding the history of the family, parental age, occupation, affected sibship, status and type of marriage, treatment if any, received by mother during pregnancy, number of spontaneous abortions, diagnosis and clinical features of the patients were recorded in a proforma.

Blood samples were cultured according to the standard technique of Moorhead et al. (1960). Heparinized plasma or whole blood was inoculated with culture medium supplemented with a mitogenic agent, Phytohaemagglutinin (PHA, Welcomes, UK) to produce a short lived burst of mitosis. Maximum response usually occurred by the third day.

STERILIZATION PROCEDURES

The procedure for the preparation of glasswares and other apparatus were aimed at prevention of contamination with noxious chemical substances. All
glasswares were placed in nontoxic soap water, followed by washing in running water for 24 hrs. These were then rinsed in distilled water. Before sterilization, these were packed in aluminium foil and then autoclaved at 15 pound pressure/second for 30 minutes.

PREPARATION OF TISSUE CULTURE MEDIUM

The medium was prepared every month for setting lymphocyte culture. The procedure for preparing medium is given below:

10 gms RPMI (Roswell Park Memorial Institute Medium) 1640 media with Hanks base (powder form) added in 950 ml of sterilized triple destilled water in sterile flask and mixed gently.

About 20-30 ml (3-5%) of sterile sodium bicarbonate solution was added to the medium and pH was adjusted at about 7.0 to 7.2 (light red-brown colour). The media were sterilized by filtering through millipore filter (Pore size 0.22μ). It was then dispensed in 100 or 250 ml of sterile bottles and corked tightly for storage at 4°C.

LEUCOCYTE CULTURE TECHNIQUE

The technique used in the present investigation gave excellent well-scattered somatic metaphase. The
various steps of the technique are as follow:

1. 1 ml of venous blood under complete aseptical condition was collected and transferred into a tube containing 100 units of heparin and mixed gently to avoid clotting.

2. About 0.3 ml of whole blood was added into a sterile 20 ml screw capped culture bottle (universal container) containing 5 ml of tissue culture medium (RPMI-1640) with Hank's base supplemented with 1.00 ml (20%) of fetal calf serum and 0.1 ml of phytohaemagglutinin (Wellcome, UK or Difco, U.S.A.).

Culture bottles were incubated at 37°C for 72 hours and the bottles were shaken every 24 hours. Colchicine (Gibco, 25ug/ml) was added (0.15 ml) to each culture three hours before removal from the 37°C incubator. The whole content of culture bottle was transferred into centrifuge tubes and spun at 1000 rpm for 5 minutes. The supernatant fluid was removed and 10 ml of prewarmed freshly prepared potassium chloride solution (0.075M) was added to the culture and reincubated at 37°C for 10-15 minutes. The tubes were centrifuged at 1000-1500 r.p.m. for 5 minutes and the supernatants were discarded. Afterwards, the cells were
fixed by adding freshly prepared chilled fixative (3:1 ratio of methanol and acetic acid). Fixative was added drop by drop with gentle shaking. After adding 1 ml of fixative and mixing the contents, the volume was increased upto 8-10 ml by adding more fixative.

The tubes were left in a refrigerator or at room temperature for half an hour in order to fix the cells. Afterwards, the cells were resuspended in 5 ml of fresh fixative and centrifuged for 5 minutes, in order to obtain the colourless material for making slides. The centrifugation was repeated 5 to 6 times.

PREPARATION OF CHROMOSOME SLIDES

The slides were soaked in chromic acid or absolute alcohol overnight. They were then washed in running water for at least one hour and rinsed in distilled water and polished thoroughly with a glass cloth.

The supernatant was completely discarded without disturbing the cell button. Finally, 0.5 to 0.75 ml of freshly prepared fixative was added. The final concentration of the cell suspension was adjusted depending on cellular content. 2-3 drops of the cell suspension were dropped with pasture pipette on the dry and pre-cleaned slide placed at a 30° angle from a
distance to facilitate better spreading. Three to four successive drops of a diluted cells suspension were evenly poured on the slide. This produced excellent spreading. The slides were both air and flame dried. These were then coded and stored for further study. Giemsa solution (1-2%) was used at pH 6.8 to 7.00 for staining the slides.

C-Banding

In order to identify the position of centromere, satellite associated acrocentric chromosomes and to demonstrate dicentric chromosomes C-banding was done. In the present investigation, C-band technique of Sumner (1972) was used which is briefly described below:

1. Both air dried and flame dried chromosome slides were left for 7-10 days before banding technique was employed.
2. The slides were treated with 0.2N Hydrochloric Acid (HCl) for one hour at room temperature.
3. The slides were throughly rinsed (at least two changes) in distilled water and air dried.
4. They were incubated in 5% aqueous solution of barium hydroxide (BaOH$_2$) at 60°C for 3-7 minutes (depending upon the age of slides).
5. It was followed by washing in distilled water in
order to remove the precipitate of barium hydroxide. They were then air dried and put in 2xSSC (0.88 mg sodium citrate + 1.75 mg sodium chloride in 100 ml D.W.) in coplin jar for about an hour at 60°C and then rinsed several times in distilled water, the staining was done in 5% buffered Giemsa solution at pH 6.8 at least for 10 minutes.

SISTER CHROMATID DIFFERENTIAL STAINING

The sister chromatid differential staining was done in some cancer patients. The following technique was used.

SCEs Studies in vitro

1. 0.3 ml of heparinized blood was added to culture tube containing 5 ml of RPMI-1640 culture medium supplemented with 15% fetal calf serum and 0.1 ml of PHA.

2. 5 μg/ml of 5-bromodeoxyuridine (BrdU, Sigma, U.S.A.) was added to it at the initiation of culture.

3. The cultures were allowed to grow in complete dark for 72 hours by which time the majority of cells have transversed through two DNA synthetic
phase.

4. After harvesting the culture and making the slides, 8-10 days old slides were treated for SCE.

STAINING FOR SCES

3-4 drops of Hoechst 32358 (50 ug/ml) were poured on the slides and covered with coverglass.

The slides were left for 8 minutes at room temperature and then rinsed in distilled water followed by drying in air.

These were treated in 2xSSC by putting 3 drops of 2XSSC on the slides and covered with coverslip. These were exposed to sharp sunlight in a moist condition for 30-60 minutes. Then the cover glasses were removed from the slides and rinsed twice in distilled water and dried. Giemsa stain (2% pH 6.8) was used for staining the slides for 5 minutes and then observed under the light microscope (C.Z. Jenaval).

PHOTOGRAPHY AND KARYOTYPING OF CHROMOSOMES

The first preliminary step in chromosome analysis was the eye karyotype. The knowledge of the classification of normal and abnormal human chromosomes is required at the onset. A metaphase spread was
examined with oil emersion magnification (about 1000X) on a standard light microscope. The analysis of chromosomes was done in some of the cases by rough drawings which were usually important as a useful screening procedure for detecting abnormalities in chromosome number or gross morphologic abnormalities. Several metaphase cells were eye karyotyped. The counting of metaphases was routinely made by visual observations followed by counting from the photographs of the selected metaphases. Efforts were made to avoid any broken, clumped and overspreaded chromosomes to be photographed unless to show some gross aberrations such as, dicentrics, breaks or gaps which might be seen in one or a few chromosomes only. Karyotyping or arrangement of chromosomes was done according to the classification proposed by Patau (1961).

1. As many as 30 to 50 metaphase cells were counted on each standard slides.
2. More metaphases were counted and photographed when mosaicism of chromosomes was existent.
3. To determine the characteristic of the aberrations for each chromosome in early metaphase, mid-metaphase and late metaphases, 6 to 10 high quality and well spread chromosomes
were photographed for the better objectivity of chromosomal abnormalities found.

4. Chromosomes in the photographs were divided into four regions in a camera image with pencil lines (Boddington et al., 1965) for avoiding any bias in counting.