STUDIES IN NORMAL AND ABNORMAL HUMAN EMBRYOGENESIS

by

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I N D E X

INTRODUCTION

During the intrauterine stage of life, a heavy toll is exacted from the Human race in the form of abortion and prematurely terminated pregnancy. To assess the wastage from abortion in quantitative terms that are anywhere near the truth is far from possible, as denoted by Tietze (1953). However, indirect and fragmentary evidence suggests that as high as one-fourth to one-third of all gestations may be lost during the first twenty weeks of pregnancy, and another 10% later therein (Volaoras, 1953). Such is certainly a high loss, and efforts to minimise it have not been as successful as those spent in the neonatal and later stages of life.

To investigate the problem, it was clear that the maximal possible information about the various aspects of abortion had to be collected. Over many decades it gradually became known that certain fertilized ova were lost on account of lack of a favourable environment, whereas others carried intrinsically within them the seeds of their destruction. Perhaps the one happy aspect about abortion was its "filter" action, excluding from life - at an early stage - those human beings affected with such malformations as to make future life impossible or

unbearable. The thalidomide tragedy caused quite a stir even in the lay mind, when it was realised that some of these malformations were actually iatrogenic and, to say the least, preventable. Then, fresh impetus was given to the research in congenital malformations. Meanwhile modern methods of tissue culture permitted the real probing of human chromosomology, a subject that very soon had its bearings on certain human diseases and congenital abnormalities. Few are the examples in the history of Medicine when so significant information was obtained in such a short time.

With all this in mind the present work was planned. It is a study of the "abortion material" collected in a large hospital: The Simpson Memorial Maternity Pavilion, Royal Infirmary, Edinburgh. The study comprises certain general, anatomical, pathological and cyto-genetic aspects.

PART 1.

MATERIAL AND METHODOLOGY

The General Scheme of the Work

Ward and Theatre Sisters at the S.M.M.P. and the Gynaecological Section of the R.I.E. were requested to collect any abortion products and send them to this Laboratory. Special polythene bags in a range of sizes were used for the purpose, and sterile saline solution was supplied for use instead of the usual preservatives which would be incompatible with subsequent tissue culture. To minimise infection (the real nuisance in tissue culture), aseptic handling and immediate dispatch were necessary, but specimens arriving after working hours would be kept in the refrigerator till next morning. The specimen bags were securely tied, and each carried a label denoting the name of the patient, the ward or operation theatre as relevant and the date and time of procuring the specimen. The Sisters were briefed on the project and had a clear idea about the purpose and fate of the specimens they sent.

Upon receiving a specimen it was registered in a "Daily Entry Book" and given a serial number, always to be referred to as the "Project number". A special folder bearing the name and Project number was designated to include all the data about each case.

The specimen was then inspected in detail. All grades from the intact sac comprising placenta,

membranes, cord and foetus to the scanty bits of evacuated material were represented. When it was decided to take tissue for culture this was done first, before contamination set in. Sterile instruments were used for the purpose, and the foetal skin was mopped with 70% alcohol before cutting into it. Portions of skin, fascia, amnion and chorion were taken and immediately put in separate screw capped bottles containing 199 solution, labelled and dispatched to the nearby culture room. A detailed morphological description of the specimen was then made, covering embryo, cord, membranes and placenta as will be detailed under Anatomical Technique. Early embryos were inspected under the dissecting microscope to reveal any external anomaly, but in embryos of three or more months a methodical autopsy could as a rule be done, unless the viscera were too digested by maceration.

As soon as tissue was taken for culture, one was free to fix the specimen in formol-saline solution, which is probably advantageous before examining the placenta, since areas of haemorrhagic infarction over the cut surface of the placenta do not otherwise show in contrast with the rest of the surface. The difficulty about the use of formalin is its weight reducing effect on tissues as discussed by Robles & Pratt (1961) and confirmed during this work. Of course the placenta and foetus could be weighed fresh

then preserved, but the weights of the internal organs would certainly change if autopsy was to be performed after a period of formaldehyde fixation.

Having covered the anatomical description, tissue biopsies were taken for the preparation of histological sections. These included representative bits from various areas of the placenta, the membranes, the umbilical cord nearest to the foetal end and the gonads (right and left). For nuclear sexing placental tissue was used as a routine, but a skin and muscle biopsy was taken when placental tissue was absent. When the material sent consisted only of a small embryo, the whole of it was sent for multiple sectioning. Biopsy specimens were fixed in 10% formol-saline solution. Sections 6mu. thick were prepared and stained with haematoxylin and eosin for the ordinary histological examination. Although often possible to read the sex-chromatin in such sections, it was necessary to use other staining techniques to be described later.

At a later date the clinical notes of the patient were extracted and recorded on a special form. This included the name of the patient, age, marital status, dates of admission and abortion or operation, the medical, menstrual and obstetric histories and as many details as possible about the present pregnancy with special note of the nature and time of onset of the presenting symptoms and any possible aetiological factors. This sheet was then included into the folder.

ANATOMICAL TECHNIQUE

The steps of anatomical examination were derived from those described by Javert (1957), Potter (1961) and Hertig (1961).

The Foetus

External Examination:

The external surface of the foetus was carefully inspected for evidence of maceration, cyanosis, injury and skin lesions. Gross external appearances such as the excessive oedema of hydrops or the shrivelling up of a long retained dead foetus were noted. The orifices were examined for patency, exudations or other abnormalities. The state of the umbilicus was noted. A careful search for any congenital malformations was made, including regional examination of the head and neck, the chest, abdomen, back, limbs, fingers and toes and the ano-genital region. The oral cavity was examined and a plastic catheter was passed orally into the stomach to establish oesophageal patency but it was often advantageous to defer this step until the abdomen was opened. The general appearance of the face was examined for possible resemblance to any of the conditions having characteristic facial changes such as renal agenesis. The position and shape of the eyes, nose and ears was noted. The degree of hair and vernix coverage were noted, as well as any results of the mechanical

forces thought to act on the foetus in the uterus or as it passed through the birth canal.

Weights and Measurements:

The foetus was weighed in whole. When autopsied without formalin fixation the thymus, liver, kidneys, suprarenals and lungs were separately weighed when possible. Formalin fixation was found to reduce the weight, and when the foetus had been fixed in formalin before autopsy organ weights were not done.

Three measurements were taken as a routine, viz. the crown-rump (CR), crown-heel (CH) and heel-toe (HT), and recorded in centimeters.

Autopsy:

Head - The tissues beneath the scalp, the skull bones and sutures were inspected before opening the skull. The inside of the skull was examined by cutting a large window in each parietal bone so that the tentorium cerebelli and the falx cerebri remained intact. One blade of a pair of scissors was inserted in the most inferior portion of the suture between the frontal and parietal bones and an incision was started and carried up between the bones to the point marking the beginning of the anterior fontanelle. The dura was incised simultaneously with the skull. The line of incision then left the suture and was continued parallel and near to the superior and posterior edges of the parietal bone and as it reached the inferior margin the incision was

extended forwards for some distance. Reflection of the bone permitted examination of the brain surface. The occipital lobe of the brain was then gently lifted forwards with the handle of a knife and the falx, tentorium and the vein of Galen inspected. The bony cap was then replaced to protect the brain and the procedure repeated on the opposite side. The brain was then removed or incised in situ to examine the relative size and the contents of the ventricles. After removing the brain, the cranial floor was inspected.

Spinal cord - This was inspected after opening the spinal canal by cutting through the laminae on the right and left sides of the vertebrae and removing the central strip containing the spines.

Abdomen - A broad oval flap of skin and anterior abdominal wall was removed, from the xiphisternum along the costal margins and flanks to the pubic region. The urinary bladder was dissected free, and the urachus and umbilical vein severed at the umbilicus. The under surface of the diaphragm was inspected for the presence of any gaps. The liver was inspected and before its removal the patency of the bile ducts was ascertained by opening the duodenum and applying gentle pressure over the gall bladder to expel bile. The spleen was inspected and removed. The gestro-intestinal tract was then followed in continuity from the rectum to the stomach, and then it was removed, leaving the kidneys and adrenal

glands in full view on the posterior abdominal wall. The ureters were examined and traced down to their insertion in the bladder. The bladder was gently squeezed to establish patency of the urethra by expression of urine. The genital system was then examined and each gonad taken out for histological examination.

Thorax - The thorax was opened by cutting through the rib cage upwards from the mid-axillary line to the costo-clavilcular junction. Care was exercised not to injure the lungs with scissors. The diaphragmatic attachment below had to be cut, and the upper part of the sternal plate had to be dissected off the thymus. The thymus was dissected off and removed, without injuring the innominate vein if possible. The pericardium was then removed as completely as possible and the chambers of the heart and the great vessels examined and normal anatomy ascertained. The heart could then be removed by successive severing of the inferior vena cava, pulmonary veins, superior vena cava, pulmonary artery and aorta. The interior of the heart was then examined, first making an incision from the superior vena cava, through the atrial wall and inferior vena cava, through the tricuspid valve, along the right side of the ventricular septum, around the apex, up the anterior surface of the right ventricular wall and ending at the cut end of the pulmonary artery. The same procedure was repeated on the left side of the heart

beginning the incision at the pulmonary veins, down through the mitral valve, along the left side of the ventricular septum and out the aorta. The chambers, valves and septa were thus adequately examined. The lungs were then examined, then the trachea dissected off the oesophagus and opened for inspection.

The Umbilical Cord

The cord was inspected to discover such anomalies as abnormal loops, knots, excessive torsion, stricture, oedema, haematoma, cysts, loss of normal spiralling and abnormal tone or colour. The length of the cord was measured to compare it to that of the foetus. The cut section was inspected for the number of vessels, and a segment from near the foetal end was routinely taken for histological examination.

The Placenta and Membranes

The significant features to be recorded when examining the placenta were weight, size, shape, colour, consistency and completeness of the membranes with site of rupture. On the foetal surface were noted the insertion of the cord, the attachment of the membranes, the condition of the foetal vessels and the presence of

subchorionic haematoma or opaque fibrin deposits. On the maternal surface were noted the intactness of the surface, recent or old blood with its location and extent, calcarious deposits, marginal haemorrhage and vesicular changes in the terminal villi. Multiple parallel sections were then made and the cut surface inspected for infarction and intervillous thrombosis. Tissue blocks were then collected for microscopic examination including the margin with maternal and foetal surfaces, healthy placenta, any pathological lesion and a roll of membranes.

SEX-CHROMATIN STAINING TECHNIQUES

I The Feulgen Staining Technique for Tissue Sections

Culling (1957)

Method:

(1) Immerse the sections in xylol, absolute alcohol and through descending alcohols down to water.

(2) Rinse in cold N/1 hydrochloric acid.

(3) Transfer to N/1 hydrochloric acid at 60°C for 10 minutes. (Pre-heat the acid to 60°C).

(4) Rinse the section in cold hydrochloric acid. Rinse in distilled water.

(5) Transfer the section to Schiff's reagent for 30 - 90 minutes.

(6) Rinse in sulphite solution 1 for 1 minute.

(7) Transfer to sulphite solution 2 for 2 minutes.

(8) Transfer to sulphite solution 3 for 2 minutes.

(9) Rinse well in distilled water.

(10) Counter stain with 1% aqueous light green for a few seconds.

(11) Dehydrate, clear and mount.

Expected Results:

D.N.A. Red-reddish purple.

Other tissue constituents: green.

Preparation of Schiff's Reagent:

(1) Dissolve 1g. of basic fuchsin in 200 ml. of boiling distilled water, in a stoppered 1 litre flask.

(2) Shake the flask well for 5 minutes.

(3) Cool to exactly 50°C, filter and add 20 ml. of N/1 hydrochloric acid to the filtrate.

(4) Cool further to 25°C and add 1 g. of sodium or potassium metabisulphite.

(5) Store in the dark for 18 - 24 hours.

(6) Add 2 g. of activated charcoal.

(7) Shake for 1 minute.

(8) Filter to remove the charcoal and store at 0° - 4°C.

Preparation of sulphite solution:

Method:

(1) Place 135 ml. distilled water into a flask.

(2) Pipette in 7.5 ml. of 10% potassium metabisulphate.

(3) Shake well to ensure good mixing.

(4) Add 7.5 ml. N/1 hydrochloric acid. Shake well again to mix.

(5) Distribute into three Coplin jars.

II Staining of Paraffin Sections by a Combination of the Feulgen and Thionin Methods

Klinger (1957)

Method:

(1) Bring the sections down to water.

(2) Rinse in 1N Hcl. at room temperature for a few minutes.

(3) Transfer to 1N Hcl. at 60°C for 5 minutes.

(4) Rinse again in 1N Hcl. at room temperature.

(5) Wash thoroughly in several changes of distilled water.

(6) Transfer to the thionin buffer solution at pH 5.5 for 15 minutes.

(7) Rinse off the excess dye in 70% alcohol.

(8) Immerse for 15 minutes in 95% alcohol ("blind" differentiation).

(9) Dehydrate, clear and mount.

Preparation of the Thionin buffer solution:

Requirements:

(1) Saturated solution of thionin in 50% alcohol (filtered).

(2) Michaelis buffer:

9.714 gm. sodium acetate ($3H_2O$)

14.714 gm. sodium barbiturate

500 ml. distilled water (CO_2 free)

(3) 0.1 N HCl

Method:

Mix 28 ml. buffer solution with 32 ml. 0.1N HCl and bring up to 100 ml. with thionin solution.

The pH should be 5.7 ± 0.2.

III The Cresyl Echt Violet Stain for Cellular Smears (Buccal Smears, Amniotic Fluid Centrifugate) and Amnion Mounts

Moore and Barr (1955)

Method:

(1) Fix the smears immediately in 95% alcohol (Graham, 1957) for a minimum of 30 minutes.

(2) Rehydrate through 50% alcohol to water.

(3) Place in a Coplin jar containing 0.5% aqueous solution of Cresyl echt violet (Coleman and Bell)* for 5 minutes.

(4) Rinse quickly in tap water to remove excess stain.

(5) Dehydrate through 95% alcohol and absolute alcohol.

(6) Clear in two changes of xylol.

(7) Mount in D.P.X.

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*Cresyl echt violet (Coleman and Bell) obtained from C.T. Curr Ltd.

TECHNIQUE OF TISSUE CULTURE AND PREPARATION OF SLIDES FOR EXAMINATION OF THE CHROMOSOMES

Harnden (1960).

Section A Preparation of Primary Culture

Requirements: (for 2 specimens)

(1) One Petri dish and cover, and one watch glass - for each specimen.

(2) Two Petri dishes and covers - enough for 2 specimens.

(3) Fine scissors; fine forceps.

(4) Pasteur pipettes; rubber teat.

(5) Infant feeding bottles (2 for each specimen); silicone rubber bungs.

(6) Cock plasma; C.E.E.: solution 199; human AB serum; 5% CO2 in air.

(7) Large bunsen; tripod; 100 ml. beaker; marking pencil.

Procedure: (for 2 specimens)

(1) Tip contents of specimen bottles (i.e. tissue in 199) on to the watch glass which is left lying in the Petri dish. Cut this into small fragments, 1 - 2 mm. diameter - as many pieces as possible (c. 20-30 if possible).

(2) To the other 2 Petri dishes add several (c. 12) blobs of cock plasma in one, and an equal number of blobs of C.E.E. to the other. Replace covers on each dish while not in use.

(3) Using a sterile Pasteur pipette, draw up one or more (up to 4) pieces of tissue in 199. The cover of the Petri dish which has contained the tissue has been inverted and lies beside the dish. Squirt out the contents of the pipette on to this cover and aspirate off the 199, leaving behind the fragments of tissue.

(4) Suck up a blob of C.E.E. in pipette, and transfer it to a blob of cock plasma. Mix together by aspiration and expulsion in the pipette, and finally draw the mixed "C.E.E. - plasma" into the pipette.

(5) Transfer this "C.E.E. - plasma" mixture to the tissue fragments on Petri dish cover, and then draw up this "tissue - plasma - C.E.E." mixture into the pipette.

(6) Insert pipette into culture bottle, and place each piece of tissue separately, sweeping around each a circle of plasma.

(7) Repeat the process until each bottle contains 12 - 16 fragments. Between each of these maneouvres, draw up a small quantity of boiling water to remove excess plasma and so avoid clotting. Then draw up a small amount of 199 from Petri dish cover to remove this water.

(8) Leave bottle for 15 - 20 minutes for plasma to clot.

(9) Make up required quantity of growth medium. (See

section B., procedure 6).

(10) Once clot is firmly set, add 5 ml. growth medium. Gas solution. Cork firmly. Place in incubator (medium need not be changed for 2 days).

N.B. a) From each tissue specimen, set up two parallel primary cultures.

Section B. Care of Cultures and Preparation of Subcultures

Requirements:

(1) Inverted microscope; angle-poise lamp; incubator; bench centrifuge; baskets for dirty glassware; metal test-tube rack; small bunsen; suction apparatus; 5% CO_2 in air.

(2) Pasteur pipettes; graduated pipettes (2.5 and 10 ml.); Pumpett pipette filler; infant feeding bottles; silicone rubber bungs; conical flask (50 mls.); centrifuge tubes; glass marking pencil; day book; 3 measuring cylinders (100 mls.).

(3) C.E.E., human AB serum; solution 199; solution A; $NaHCO_3$; stock x 10 Trypsin solution; mycostatin.

Procedure:

(1) Check all cultures (primary and subcultures) thrice weekly for state of growth and freedom from infection. Primary cultures are checked for state of growth and number of divisions (on inverted microscope) to assess timing of subcultures.

Subcultures are checked for presence of a good monolayer and divisions to assess timing for "cytology" or need for further subculture if growth is too heavy.

(2) Every culture must have medium changed thrice weekly.

(3) Both "cytology" and subculturing require preliminary digestion with trypsin:

i.e. following these preliminary inspections (procedure 1) one can decide how many trypsin digestions are required. Therefore one can then calculate the total quantity of growth medium which will be required that day; thus,
for each trypsin digestion - 10 mls.
for each replacement of medium - (5 mls.
for each primary culture still in cock plasma. 10 ml. for each subculture.)

(4) When trypsin digestions are necessary, it is preferable to do these first, and make up growth medium while these are incubating.

(5) Preparation of subcultures.

(a) From stock X10 trypsin solution, take $\frac{x}{10}$ mls. and place in a small conical flask, adding $\frac{9.x}{10}$ mls. Solution A. (where x = total volume of trypsin solution which will be required).

(b) Aspirate growth medium from culture bottles.

(c) Add 10 mls. trypsin solution.

(d) Incubate for 10-20 minutes. (certainly no longer than 20 mins.)

(e) During this incubation, prepare growth medium (see procedure 6)

(f) Add 9 mls. growth medium to each subculture bottle required; number each bottle appropriately.

(g) Prepare centrifuge tubes and silicone bungs, numbering each appropriately.

(h) Decant contents of 'digested' culture bottles into centrifuge tubes and stopper.

(i) Spin for c. 5 - 10 mins. at c. 500 r.p.m. (or until cells are well packed down).

(j) Decant supernatent.

(k) Add c.1 ml. growth medium with Pasteur pipette to plug of cells in centrifuge tube, and bring cells into suspension by gentle aspiration with pipette.

(l) Add either whole or part of these cells to the prepared subculture bottle containing growth medium. Gas each bottle. Stopper firmly. (Usually one needs all the cells from plasma clots, but only part from subcultures.

(m) Gas all new bottles.

(6) Make up growth medium - volume as previously calculated (procedure 3).

i.e. C.E.E. - 10%) made up in 100 ml. measuring cylinder.
Human AB serum - 20%) If C.E.E. is in short supply, one may use 5% C.E.E.
Solution 199 - 70%)

Adjust pH of this medium with $NaHCO_3$ till medium is faintly blue.

(7) Change all culture media - 5 mls. for each primary culture, and 10 mls. for each subculture.
"Gassing" is only required when changing bottles, or if solution is too blue.

(8) If there is any monilial infection, add mycostatin - c. 200 ug./bottle. Use a stock solution of 1000 ug/cc., adding approximately 0.1 ml. (i.e. 1 - 3 drops) to each bottle.

PREPARATIONS FOR CYTOLOGICAL PROCESSING.

Section C.

Requirements:

(1) Culture room facilities. (See Section B.)

(2) Water bath at 37°C; deep freeze.

(3) Siliconed centrifuge tubes; conical flask (50 mls.); graduated pipettes (2 and 10 mls.); 2 measuring cylinders (25 mls.) and 2 Pasteur

pipettes; silicone rubber bungs; glass marking pencil; rubber teat.

(4) Stock trypsin solution; B.S.S.; colchicine solution; 3.8% sodium citrate; solution A; distilled water; glacial acetic acid; absolute alcohol.

Procedure:

(1) Choose subcultures where there is a good monolayer with frequent divisions.

(2) Add 0.5 ml. colchicine solution, and incubate at 37°C for 4-5 hours.

(3) Decant off medium.

(4) Add 10 ml. trypsin in solution A (section B, procedure 5(a)), and incubate for approximately 10 minutes.

(5) Meanwhile prepare:

(a) "Hypotonic solution" - i.e. 4 parts 3.8% sod. citrate to 12 parts distilled water (non-sterile), allowing 8 mls. for each culture. Prepare this in a clean non-sterile 25 ml. measuring cylinder, which is marked and kept solely for this purpose. Pasteur pipettes used for this purpose should also be marked and reserved for this use. Place in a water bath at 37°C.

(b) "Fixative" - i.e. 4 parts glacial acetic acid to 12 parts absolute alcohol, allowing

8 mls. per culture. Prepare in a similarly marked 25 ml. cylinder and Pasteur pipette. Leave at room temperature.

(6) Remove culture from incubator, and add its contents to siliconed centrifuge tubes (clean, but not sterile), and spin slowly for c. 5 minutes.

(7) Decant supernatant, and "flick" cells into dregs of fluid.

(8) Add B.S.S. (not calcium and magnesium free), approx. 10 mls., and spin for 5 minutes.

(9) Decant and again "flick" up cells.

(10) Add approximately 8 mls. hypotonic solution, and place in water bath at 37°C for 20 minutes.

(11) Remove and spin slowly for 5 minutes.

(12) Decant and add approximately 8 mls. fixative, adding it very slowly drop by drop along side of tube, to avoid clumping of cells.

(13) Keep overnight in deep freeze.

PREPARATION OF SLIDES

Section D.

Requirements:

(1) Centrifuge; incubator.

(2) Plastic beaker; Pasteur pipettes; marking pencil; rubber teats; bunsen burner;

test-tube holder; dish towel.

(3) Ice-cubes; 75% acetic acid; orcein.

(4) Ground glass microscope slides.

Procedure:

(1) Prepare a plastic beaker of iced water.

(2) Spin down cells (taken from deep freeze) for 5 - 10 mins.

(3) Meanwhile, dry slides on a clean towel, allowing 2 for each culture, number them and place in iced water.

(4) Remove from centrifuge, decant supernatant, and "flick" up cells.

(5) Add 75% acetic acid - actual volume depends on the number of cells, but usually a few drops are adequate - i.e. just enough to make a fair cell suspension, and not too much to over-dilute it.

(6) Remove slides from iced water and shake off excess water.

(7) Drop on a few drops of cell suspension, and allow it to spread (usually 2-4 drops will suffice).

(8) Remove excess by simply inverting the slide.

(9) Heat slide fairly vigorously on bunsen.

(10) Place slides in orcein solution in Coplin dish when they are reasonably cool and incubate

for approximately 2 hours (or longer does no harm).

(11) Remnant of cell suspension may be kept in deep freeze for other preparations, in case this should fail.

N.B. (a) When making slides, use a different pipette for each cell suspension.

(b) Slides are kept in a beaker of absolute alcohol with a few drops of concentrated HCl. added.

PREPARATION OF CHICK EMBRYO EXTRACT

Requirements:

(1) Absolute alcohol, B.S.S., $NaHCO_3$, penicillin, streptomycin, hyaluronidase.

(2) Beaker - 100 mls; 3 measuring cylinders - 100 mls; beaker - 500 mls; tissue grinder; 12 universal containers; Apinco tubes.

(3) Candle filter apparatus - 5% CO_2 in air, rubber tubing, 1L round flask, special glass fitting, polythene tubing, candle filter, 1L conical flask with side-arm, wire and wire-cutter, retort stand and fitting.

(4) Cotton wool, scalpel handle, beaked forceps, bunsen, dish cloth, bucket.

(5) M.S.E. centrifuge, incubator, Spinco, deep freeze, refrigerator.

Procedure:

(1) Take egg, marked side uppermost, and wipe with cotton wool soaked in absolute alcohol.

(2) Crack shell over air-space with scalpel handle, and remove shell and adjacent membrane.

(3) Keep beaked forceps in beaker of absolute alcohol, flaming points over bunsen before each usage.

(4) Pick off membrane with beaked forceps, hook out embryo by neck, and drop it into 100 ml. measuring cylinder.

(5) Repeat this process until all embryos are in cylinder.

(6) Measure total volume of embryos = x. mls.

(7) Take $\frac{5}{4}$x. mls. B.S.S., (adding to each 200 mls. bottle of B.S.S., prior to use, one bottle of $NaHCO_3$, penicillin and streptomycin).

(8) Add Embryos to tissue grinder, approximately 30 mls. at a time (i.e. "embryo aliquot").

(9) Calculate volume of B.S.S. which is proportional to this embryo aliquot. (i.e. "B.S.S. aliquot"), and add approximately 10 mls. of this B.S.S. aliquot to embryos in the grinder.

(10) Grind thoroughly .

(11) Calculate the number of universal containers which will be required to contain the combined "embryo - B.S.S. aliquot", and distribute

some of the contents of the grinder equally in these containers.

(12) Add to the grinder the remainder of the B.S.S. aliquot. Mix lightly, and add the whole to the universal containers. Stopper tightly.

(13) Repeat this process with rest of embryos and B.S.S., until all is distributed in universal containers.

(14) Spin in MSE at 2,500 r.p.m. for 30 minutes (balancing each pair of carriers and containers accurately with water.

(15) Pour off supernatant into 500 ml. beaker, and add hyaluronidase, allowing 0.002 ml. per 100 mls. embryo - B.S.S. solution.

(16) Incubate at 37°C for 1 hour.

(17) Spin at 25,000 r.p.m. for 1 hour in Spinco.

(18) Filter through candle-filter for c. 1 - 2 hours.

(19) Distribute in universal containers, and store in deep freeze.

(20) Thaw when ready for use.

N.B. (a) Spinco tubes are washed in Elgastat, but are not sterile.

Preparation of Hank's Basic Salts Solution (B.S.S.)

(1) $CaCl_2$ 1.4 g.) dissolve in 200 mls. water.

(2)

Glucose	10.0g.	place in 1L beaker and dissolve in 790mls. water.
NaCl.	30.0g.	
KCl.	4.0g.	
$MgSO_4$ $7H_2O$	2.0g.	
KH_2PO_4	0.6g.	
Na_2HPO_4 $12H_2O$	0.6g.	
(or anhydrous Na_2HPO_4	0.48g.)	

(3) Mix these two solutions.

(4) Add 10 mls. phenol red solution.

N.B. (a) Use deionised water, and highest quality analar salts.

(b) This method provides an X10 stock solution. This cannot be autoclaved and must be preserved with chloroform (3-4 mls./L)

(c) When solution is required for use, dilute 1 in 10. Usually make 100 ml. up to 1L for use. Distribute this in 200 ml. aliquots in screw-capped flat medicine bottles.

(d) These medicine bottles are autoclaved at 15 lb. per sq. in. for 20 minutes. Caps should be slackened before putting in autoclave.

(e) Before each medicine bottle is used, add 5 ml. $NaHCO_3$ solution. (i.e. contents of one Bijou bottle), plus one bottle each of penicillin and streptomycin.

(f) Stock solution is kept in a reagent bottle with glass stopper, and it will keep indefinitely.

(g) Both stock and dilute solutions are stored at +4°C.

Routine Procedures

(1) Suction Apparatus. Venturi pump is attached to a sterile flask via a permanently fixed rubber tube. Flask is fitted with 2 B.T.S. needles. The rubber tubing is attached to one of the needles, and to the other is fitted a clean (not sterile) plastic tubing, which is discarded after each use. Plastic tube in turn is fitted to a sterile Pasteur pipette.

(2) Silicone Centrifuge Tubes. Add a small quantity of Repelcote, and swirl it around for a few minutes. Remove the fluid (it can be used again). Rinse thoroughly in water thrice. Bake in oven for 1 hour at 160°C. This will last for approximately 6 months.

(3) Waxed Tubes and Pipettes. Autoclave a large covered beaker of paraffin wax. Using sterile pair of long forceps, plunge the sterile centrifuge tubes into the molten wax. Remove and invert to allow wax to drain off, and allow to cool. Store in B.T.S. tin.

Pasteur pipettes are similarly prepared.

(4) Candle Filter.

(1) - retort stand and fitting.

(2) - 1L. round flask.

(3) - special glass fitting.

(4) - (5) - polythene tubing.
wired on to glass fittings.

(6) - Candle filter.

(7) - 1L. conical flask, with side-arm, plugged with cotton wool.

(8) - 5% CO2 in air, running at approx. 5 lb./sq. in.

(9) - Wire fittings.

N.B. (a) Special glass fitting is smeared with silicone grease.

(b) (2) and (3) are washed in Elgastat but not sterile; but (6) and (7) are sterile.

(c) Glass tube in round flask should virtually reach bottom of flask.

Preparation of Glassware and Instruments

Requirements:-

(1) Deioniser; still; hot and cold water supplies; sinks (2 if possible); drying oven; steriliser; autoclave; fume cupboard.

(2) Large plastic baths (3); plastic bucket; plastic dispenser for deionised water; wire baskets; nylon brushes - various sizes; rubber gloves; aluminium foil; B.T.S. boxes; N.D.M. tins; 20 ml. plastic syringe; cotton wool; dish towel.

(3) Stergene; large filter papers; concentrated HNO_3.

Procedure:

(1) Prepare 3 basins containing stergene and warm water, distilled water, and deionised water, repectively.

(2) Assemble all dirty apparatus - glassware, instruments, tubing, rubber and silicone rubber bungs, screw tops.

(3) Remove any cotton wool plugs.

(4) Steep all this apparatus in basin of Stergene and warm water for a short time (excluding only large items of glassware and fine Pasteur pipettes).

(5) Select pipettes (not Pasteur) for separate handling first:

(a) Wash in stergene and warm water.

(b) Rinse through in warm tap water (using a rubber tube attached to tap.)

(c) Rinse in distilled water.

(d) Rinse in deionised water.

(e) Place in wire baskets, lined with a large filter paper, and dry in oven at 60°C until dry (takes 3-4 hours).

(6) Proceed with rest of glassware, and after this has been removed from basin of stergene and water, place the Pasteur pipettes in the basin to steep.

(7) Wash rest of apparatus exactly as above, using

nylon brushes to ensure adequate cleaning.

(8) Pack glassware carefully in baskets - e.g. neck downwards, Petri dishes on sides etc.

(9) After drying:

(a) Glass containers - e.g. measuring cylinders, infant feeding bottles, beakers, and flasks. Cover necks with aluminium foil. Insert cotton wool in side-arms. Bake in steriliser at 160°C for 1½ hours.

(b) Centrifuge tubes, silicone bungs - place in B.T.S. boxes - sterilise as above.

(c) Petri dishes and covers, watch glasses - place in N.D.M. tins - sterilise as above.

(d) Any vessels with screw top caps - i.e. medicine bottles, Bijou bottles, universal containers - are autoclaved.

(e) Graduated pipettes are placed in plastic containers - both pipette and containers are packed with cotton wool - and sterilised as above.

(f) Pasteur pipettes have cotton wool inserted, placed in B.T.S. boxes, and sterilised as above.

(g) Instruments are placed on wadding in B.T.S. tins, and are sterilised as above.

N.B. (1) Use rubber gloves, and wash hands thoroughly when coming out of soapy water into clean water.

(2) When glassware is dirty, give preliminary soaking in HNO_3.

(3) Needles - use plastic syringes for cleaning, or if necessary, a wire pull through.

(4) Instruments are not dried in oven, but simply dried on a clean cloth.

(5) Silicone rubber bungs, screw caps, and needles are all dried in oven.

(6) Plastic and rubber tubing is washed as above, but simply dried on top of oven in a basket.

(7) Elgastat: fill basin, first thing in morning, for washing; then keep the dispenser full.

PART 2.

STATISTICAL SURVEY

STATISTICAL ANALYSIS

Some observations concerning the material that was sent to this laboratory during this work are here presented. No attempt will be made to find out or deduce the incidence of abortion. Not all abortions are admitted to hospital, and many of these patients are not even seen by a doctor. The description of potentially abortive early zygotes that can escape clinical detection as abortions, certainly adds to the difficulty of such an attempt (Hertig & Rock, 1949; Rotella Llusia & Marin Bonchera, 1961).

For the purpose of this study abortion was considered to be termination of pregnancy before it has completed 28 weeks in utero. In this sense it corresponds to Groups I and II of the classification recommended by the World Health Organisation (1950). Such definition of abortion is accepted by the majority, but there are different views on the definition of abortion, whether this has to be based on the weight or the age of the foetus, and at what age (Sullivan, 1922; Javert, Finn & Stander, 1949). Ectopic pregnancies should not be included, but three such cases found their way into this series and were deliberately kept in. In two the material sent was a foetus with interesting congenital abnormalities, whereas the third specimen consisted of villi and blood clot.

The maternal age was known for 264 patients. Unavailable or incomplete clinical notes, as well as the few specimens sent from other hospitals with no information accompanying them, account for the missing. The age distribution of the patients in this series is illustrated in Table I, showing the number and percentage of the various 5 year age groups. For reasons of comparison, the same table gives the same data for other selected groups, viz. 500 consecutive viable deliveries at the S.M.M.P. within the duration of this work, and members of this abortion series whose abortuses were blighted ova, foetuses with local abnormalities, those with illigitimate pregnancies and those whose pregnancies were terminated on medical grounds.

A look at the table shows that on the whole abortion incidence tends to rise with rising age. If we add the first two columns together, it will be found that women under 25 years of age contribute to 37.9 per cent of the viable delivery group and 34 per cent of the abortion series. Adding the last 2 columns, women over 35 years are found to constitute 10.2 per cent of the viable deliveries, but 17.4 per cent of the abortions. This observation is in accord with that of Peckham (1936), who found that the mean age in a series of 2287 cases of abortion was 27 years 5 months, whereas that for the general clinic population was 23 years 10 months.

It will also be noted that blighted ova (intact

Group of Cases	Under 20		20 - 25		25+ - 30		30+ - 35		35+ - 40		Over 40		Total	
	No.	per cent	No.	per cent	No.	per cent	No.	per cent	No.	per cent	No.	per cent	No.	per cen
500 consecutive viable deliveries in S.M.M.P.....	33	6.6	156	31.2	148	29.6	112	22.4	43	8.6	8	1.6	500	100
Present series of abortions.....	23	8.7	67	25.37	83	31.43	55	20.83	32	12.12	14	5.30	264	100
Intact blighted ova, with no foetus or cord...	3	20.0	3	20.0	4	26.66	2	13.33	1	6.66	2	13.33	15	100
Foetuses with local abnormalities.....	5	8.93	16	28.57	17	30.35	9	16.07	7	12.5	2	3.57	56	100
Illigitimate Pregnancies.....	7	30.4	8	34.78	3	13	2	8.69	2	8.69	1	4.345	23	100
Therapeutic abortions.....	0		6	33.3	5	27.77	4	22.22	3	16.66			18	100
Habitual abortions.....	0		5	41.66	2	16.67	2	16.67	3	25			12	100

TABLE I - Maternal Age Distribution

sacs with no trace of foetus or cord) show a marked concentration at the extremes of reproductive life. Whereas the "under twenty" mothers represent 6.6 and 8.7 per cent of viable deliveries and abortions respectively, they represent 20 per cent of cases with blighted ova. The "above forty" mothers behave similarly, contributing to 1.6, 5.3 and 13.3 per cent of the 3 groups.

Studying the question of maternal age in abortion, MacMahon et al (1954) suggested that the incidence of both abortion and pathological ova tended to increase with increasing maternal age, and that it might be profitable to take the age of the mother into consideration in clinical studies of the prognosis in threatened abortion. More recently, Krone (1962) noticed similar observations about the incidence of blighted ova in relation to the age of the mother. Dekaban et al (1962) also noted the higher age incidence in the abortion group than the average for other obstetric groups.

The previous obstetric history of this series of patients is summarised in Table II. Of 291 patients 53 were having their first pregnancy. This gives an incidence of primigravidae in this abortion series of 18.2 per cent, in contrast to an incidence of 50.2 per cent of primiparae among the booked cases and 45.9 per cent among the booked and unbooked clinic patients,

according to the annual Medical and Clinical Report of the S.M.M.P. (1962). This finding almost coincides with that of Peckham for the Johns Hopkins Hospital, giving an incidence of primigravidae of 17.2 per cent in the abortion series and 54.69 per cent in the total clinic patients. The incidence of previous pregnancies (abortions or viable) for the remaining 238 multigravid patients is shown in the upper column of the table. From these figures the pregnancy order of the present abortion is at once known, and has been laid down in the middle column.

The proportion of cases who have had previous abortions other than the present one, and the number of such abortions, are illustrated in the last column of Table II, for the 286 patients in whom the outcome of previous pregnancies was known. One hundred and ninety-five cases had no such abortions, 58 had one, 22 had two, 7 had three, 2 had five, 1 had six and 1 had eleven abortions other than the present. It will be noted therefore that 31.82 per cent of these patients have aborted before, and that over 11.5 per cent of them have done so more than once.

Table III gives the time interval between the present abortion and the end of the preceding pregnancy, as well as the outcome of that pregnancy. Of 187 multigravidae the last pregnancy ended during the same year as the present abortion, during the preceding year

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total
Total pregnancies (viable and abortions other than the present)																			
No. of mothers	53	57	64	44	27	18	11	12	2	1		1					1		291
%	18.21	19.58	21.99	15.12	9.28	6.18	3.78	4.12	.68	.34		.34					.34		
Pregnancy order of present abortion																			
No.		53	57	64	44	27	18	11	12	2	1		1					1	291
%		18.2	19.58	21.99	15.1	9.28	6.18	3.78	4.12	.68	.34		.34					.34	
Abortion incidence (excluding the present one)																			
No. of mothers	195	58	22	7		2	1					1							286
%	68.18	20.28	7.69	2.45		.7	.35					.35							

TABLE II. Obstetric pattern of the series.

Interval between present and preceding pregnancy	No. of cases	Outcome of preceding pregnancy		Abortion incidence in preceding pregnancy per cent
		viable	abortion	
Both same year	10	6	4	40
1 year before	62	38	24	38.8
2 years	44	36	8	31
3 years	25	19	6	
4 years	9	8	1	
5 years	9	6	3	
6 years	6	4	2	
7 years	3	2	1	
8 years	5	4	1	
9 years	5	5	0	
10 years	2	1	1	
11 years	1	1	0	22.53
12 years	0	0	0	
13 years	2	2	0	
14 years	1	1	0	
15 years	0	0	0	
16 years	1	1	0	
17 years	2	1	1	
TOTAL	187	135	52	27.8

TABLE III. The time interval since the preceding pregnancy and the outcome of that pregnancy.

in 62 and during the year before in 44. In 71 cases more than 2 clear years intervened between the present abortion and the end of the pregnancy preceding it, and in that sense they had qualified to be considered to be cases of secondary infertility until they had their last pregnancy, with an incidence thereof of 38.39 per cent. Considering the outcome of the preceding pregnancy, in 135 patients it was a delivery after the age of viability, whereas in 52 patients, i.e. 27.8 per cent it was an abortion.

An interesting relation is noticed to exist between this time interval and the preceding pregnancy terminating in abortion. In the 10 cases where the 2 pregnancies ended in the same year, the preceding one ended in abortion in 4 cases, that is in 40 per cent. If we combine together the cases when the preceding pregnancy ended during the same year as the present one and the year before it, we find that in 72 cases the preceding pregnancy ended in abortion in 28 or 38.8 per cent. When both ended during the same or the previous 2 years, we find that in 116 cases the preceding pregnancy ended in abortion in 36 or 31 per cent. When, however, more than two clear years intervened, we find that the preceding pregnancy ended in abortion in 16 out of 71 cases, i.e. 22.53 per cent only. These figures together with the high incidence of multigravidity in the series, strongly suggest an association between high

fertility, close spacing of pregnancies and a high abortion rate.

The nature of the first abnormal symptom to herald the abortion process is shown in Table IV, as well as the duration of time between its onset and the completion of abortion. Therapeutic abortions were excluded, and a previous episode of threatened abortion could be considered as the earliest abnormal symptom. Out of 231 cases, 138 (59.74 per cent) had bleeding as the earliest abnormal symptom, 16 (9.3 per cent) had lower abdominal colicky pain and back-ache, 72 (31.16 per cent) started in both bleeding and pain beginning simultaneously and 5 (2.16 per cent) started with other symptoms. These were premature rupture of the membranes with draining of liquor in 3 cases, hyperpyrexia in 1 case that was a criminal abortion, and severe hypertension in early pregnancy in 1 case, although of course that was a sign and not a symptom.

When bleeding was the presenting symptom (138 cases), abortion occured in one day in 16.66 per cent of cases and in one week in 58.66 (16.66 + 42) per cent of cases, whereas 19.56 per cent took over a week up to a month, and 21.73 per cent took longer than one month to abort.

When pain was the earliest symptom, half the cases aborted within one day and by the end of the first

Earliest symptom		Time interval till completion of abortion				Total for each symptom	Per cent of
		during same day	1d - 1w	8d - 1m	over 1m		
Bleeding	No.	23	58	27	30	138	59.74
	%	16.66	42	19.56	21.73		
Pain	No.	8	5	2	1+	16	6.93
	%	50	31.25	12.5	6.25		
Bleeding + Pain	No.	50	18	3	1+	72	31.17
	%	69.44	25	4.33	1.38		
Other symptoms*	No.	1	3	1	0	5	2.16
	%	20	60	20	0		
TOTAL						231	100

* Rupture of the membranes in 3, hyperpyrexia in 1 and hypertension in 1.

\+ Ectopic pregnancy terminated by laparotomy.

TABLE IV. The earliest abnormal symptom and the subsequent time interval till abortion.

week 81.25 (50 + 31.25) per cent had aborted. Twelve and a half per cent took over a week up to a month, and only one case (6.25 per cent) took over a month; it was a case of ectopic pregnancy terminated by laparotomy and should therefore have been excluded rather than included in this group. When abortion presented with simultaneous pain and bleeding, 69.44 per cent of cases aborted within the first day, and by the end of the first week 94.44 had aborted. The remaining minority of 4.33 per cent took over a week but within a month, while only one case (1.38 per cent) had not ended after a month and this was again a case of ectopic pregnancy treated by laparotomy.

It is evident therefore that when the presenting symptom is pain, the abortion process is expected to be brought about more promptly than the case with bleeding, whereas an onset with simultaneous pain and bleeding will usually signify abortion in the first or the few following days.

All three cases presenting with rupture of the membranes aborted within the first week, one during the first day and the others on the fifth and sixth days. The pyrexic case aborted on the second day after the onset. In the last case hypertension was discovered 2 weeks after missing her period, and she aborted spontaneously two weeks later.

Table V indicates the time interval in weeks

between the first day of the last menstrual period and the completion of the abortion, in other words the menstrual age of gestation whenever known, both for the series as a whole (the dates were known in 271 cases) and in selected groups viz. therapeutic, missed and habitual abortions and abortions complicating illigitimate pregnancies. It will be seen that the top 4-week period is the 8-12 for the whole series and therapeutic abortion, whereas for missed abortion it is the 16-20 week period. In the whole series the majority of abortions (64.54 per cent) have occurred by the end of the fourth lunar month, and this also applies to therapeutic abortions (72 per cent); but a full 81.81 per cent of missed abortions are completed after the end of the fourth lunar month.

A simplified classification of abortion into spontaneous, therapeutic and missed is given in Table VI, with the incidence of each in the 308 cases that constitute the whole series. The "spontaneous" group was made to include the "spontaneous" and "operative" groups in Peckham's classification. These were cases giving no history of interference and where completion of the abortion occurred without or with operative aid respectively. The term therapeutic abortion is self explanatory. Missed abortion was diagnosed whenever it seemed clear that a month or more had elapsed between the death of the product of conception and the actual

Menstrual age	Whole series		Therapeutic		Missed		Habitual		Illigitimate	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total Total	271		25		22		12		23	
below 4	2	.73	0	0	0		0		0	
4 - 8	21	7.75	3	12	0		2	16.66	2	8.69
8 - 12	96	35.4	10	40	2	9.09	4	33.33	5	21.73
12 - 16	56	20.66	5	20	2	9.09	1	8.33	5	21.73
16 - 20	35	12.91	4	16	9	40.91	1	8.33	4	17.39
20 - 24	38	14	1	4	6	27.27	4	33.33	3	13.04
over 24	23	8.48	2	8	3	13.63	0		4	17.39

TABLE V. Gestation age in weeks from the first day of the last period for the whole series and for various selected groups.

	Number	Per cent
Spontaneous	257	83.44
Therapeutic	26	8.44
Missed	25	8.12
TOTAL	308	

TABLE VI. Type of abortion.

miscarriage.

The diagnosis of missed abortion is not, however, an easy one. Many cases escape clinical detection, since the process may first present as abortion. It can be said with certainty that death of the foetus and its retention in utero for 4 or more weeks occurs with much greater frequency than is generally thought. To get an approximate estimate of the magnitude of this fact, an attempt was made to assess the age of a group of foetuses first by calculation from the menstrual dates and again by inference from the foetal measurements, excluding therapeutic abortions. These data were available for a group of 137 foetuses, shown in Table VII as classified into 4 week age groups both by menstrual dates and by foetal length. The crown-rump (CR) length was taken as the parameter, and the age assessed according to the table given by Arey (1954). The results were plotted in Diagram 1, to show the clear shift to the left when the age is determined by foetal length, as expected if there were many instances of foetal death occurring a long time before abortion. This can be safely said to be so, but in no way can the method be claimed as an accurate estimate. Menstrual dates can be misleading in either direction, and in 9 instances the foetal age assessed by CR length was more than the menstrual age, probably due to the apparent last normal period being actually an unrecognised episode of threatened abortion.

Estimated age in weeks		below 4	4 - 8	8+ - 12	12+ - 16	16+ - 20	20+ - 24	over 24	Total
By menstrual dates	No.	0	3	14	32	30	35	23	137
	%		2.19	10.22	23.35	21.89	25.54	16.78	
By CR length	No.	7	8	13	34	38	23	14	137
	%	5.1	5.84	9.49	24.81	27.73	16.78	9.49	

TABLE VII. Age distribution of the same group of 137 foetuses as calculated from menstrual dates and CR length.

Estimated age		below 4	4 - 8	8+ - 12	12+ - 16	16+ - 20	20+ - 24	over 24	Total
By dates	No.	0	0	1	2	9	4	3	19
	%	0	0	5.26	10.53	47.37	21.05	15.79	
By CR length	No.	2	3	2	10	2	0	0	19
	%	10.53	15.79	10.53	52.63	10.53	0	0	

TABLE VIII. Age distribution of foetuses of 19 missed abortions by dates and CR length.

Diagram 1.

Age distribution of 137 foetuses as estimated by menstrual dates and as calculated from the CR length.

Diagram 2.

Age distribution by dates and by estimation from CR length, for 19 foetuses from cases of missed abortion.

The statistical basis of the method itself is also not very sound, for whereas foetus of eg. 16 and 20 weeks are included in the same age groups, those of 20 and 21 weeks are put in separate categories. Moreover, discrepency of ages might be due to pathological underdevelopment of a living foetus, though this is probably an unimportant factor. In the group of therapeutic abortions the age was the same as assessed by both methods, and, excluding ectopic pregnancy and intrauterine death, the two curves practically coincided. When, however, 19 cases of missed abortion with available dates and measurements were considered (Table VIII), the discrepency was much more exaggerated, as depicted in Diagram 2.

There were 26 therapeutic abortions, 8.44 per cent of the total. Maternal age is illustrated in Table I, and the gestation age in Table V. In this group were included the 3 odd ectopic pregnancies treated by laparotomy and products sent. The indications for terminating the pregnancy are summarised in Table IX. Nine cases had their pregnancies terminated on psychiatric grounds, 3 of whom were single girls. The single girls were primigravidae, but all the rest were multiparae and two were grande multiparae. Three had vaginal evacuation and six had abdominal hysterotomy, with tubal ligation for sterilization in 4 of them. Three cases

had had Rubella earlier during pregnancy and their pregnancies were terminated by abdominal hysterotomy at 20 and 14 weeks, and by vaginal evacuation following the application of laminaria tents at 11 weeks. Only one foetus was adequately examined revealing no abnormality, one was not available and the third was received in little bits. Two patients had Rhesus isoimmunization problems and were terminated at 16 and 26 weeks by abdominal hysterotomy and by oxytocin drip respectively. The first was a para (3+0), all whose children had been affected and successfully treated by exchange transfusion. Her foetus and placenta were normal to examination. The second had had 3 deliveries and 1 abortion with only the first baby living after exchange transfusion. At 24 weeks she aborted after an oxytocin drip and 5 days later tubal ligation for sterilization was carried out. Hers was a missed abortion, producing a shrivelled foetus with imperforate anus and various skeletal malformations. One case had vaginal evacuation for the history of recurrent pre-eclamptic toxaemia, and one case of severe hypertension (para 2+0 aged 40) was treated by abdominal hysterotomy and tubal ligation. One case of pulmonary tuberculosis (para 4+0) was treated by abdominal hysterotomy. A para 5+0 patient had abdominal hysterotomy at 14 weeks for recurrence of breast carcinoma. One case diagnosed as intrauterine death of the foetus was treated by abdominal

hysterotomy; the foetus had right hydro-ureter and hydropelvis, and the condition of 'placenta extra-chorialis' was found.

The 3 cases of ectopic pregnancy were treated by laparotomy at 9, 12 and 9 weeks. Although not strictly abortions, the specimens sent were listed amongst the series for two reasons; first to supplement a study of sex-chromatin in ectopic pregnancies that will be mentioned at length in Part IV of this work, but second and mainly because of interesting congenital abnormalities born by 2 of the foetuses; the third foetus was not available. A foetus had a tail process, and another had phokomelia and a cranial vault gap with early encephalocele. Regarding the previous obstetric histories of these patients, they were para 1 + 0, para 2 + 0, and para 1 + 2 with previous ectopic pregnancy in the other tube. Table X summarises the modes of termination.

The series included 11 cases of habitual abortion, to which could be added cases 1 and 3 of the ectopic pregnancies giving a total of 13 habitual aborters. By habitual abortion was meant the occurrence of 3 consecutive abortions including the present one. The age incidence of the mothers is shown in Table I and gestation age in weeks in Table V. The number of such abortions was 3 in seven cases, 4 in two, 7 in one and

Psychological grounds	9
German measles early in pregnancy	3
Rhesus iso-immunization	2
Hypertensive disease	2
Pulmonary tuberculosis	1
Recurrence of breast carcinoma	1
Intrauterine foetal death	1
Ectopic pregnancy	3
Indication unknown (from other hospitals)	4
TOTAL	26

TABLE IX. Indications for therapeutic abortion.

Abdominal hysterectomy	16
of which 5 were sterilized by tubal ligation	
Vaginal evacuation	5
of which 1 was preceded with laminaria tents	
Pitocin drip	1
followed 5 days later by tubal ligation	
Laparotomy and salpingectomy (ectopic)	3
Method unknown to us	1
TOTAL	26

TABLE X. The method of termination in therapeutic abortion.

one case had 12 consecutive abortions and no viable pregnancies. Five cases however had no viable pregnancy, three had 1, four had 2 and one had 4 viable pregnancies. The term primary habitual abortion was coined by Javert, Finn and Stander (1949) to define those cases where the consecutive abortions started with their first pregnancy, in distinction to secondary habitual abortion where one or more viable pregnancies preceded. The incidence of primary habitual abortion in this series is therefore 38.46 per cent. The 5 primary habitual aborters had 24 pregnancies in all. The 8 secondary habitual aborters had 55 pregnancies, only 15 of which were viable.

Twenty-three of the abortions followed an illigitimate pregnancy. Their age distribution is shown in Table I. Over 30 per cent of them were under 20 years of age. The gestation age is depicted in Table V. Their civil status is represented in Table XI. Three of them had their pregnancies terminated on psychiatric grounds. In no case was criminal interference admitted, but it was clinically suspected in 2 cases, and one case had applied for termination on psychological reasons shortly before abortion but her request was refused by the psychiatrist. The group contained one set of twins, the mother and her fiance each was a twin. In one case the products consisted of a blighted ovum in the form of an intact sac containing no trace of embryo or umbilical cord. Sixteen foetuses were available, 8 of which were

Single.....	15
Recently married while pregnant....	4
Separated from husband.....	3
Widowed.....	1
TOTAL.....	23

TABLE XI. Civil status of illigitimate pregnancies.

macerated. Seven foetuses were found to have some form of congenital malformation including one of the twins.

During the period of this work 202 cases of illigitimate pregnancy were delivered at or near term at the S.M.M.P., amongst some 4000 deliveries. They included 2 sets of twins and only one case of malformation (anencephaly) but of course internal abnormalities compatible with life would have passed unnoticed. Eighty-four mothers, 41.58 per cent, were aged below 20 years.

The incidence of illigitimacy therefore is 7.46 per cent of the abortion group and about 5 per cent in term deliveries. The general incidence for Scotland is 6.9 per cent of live born infants. Of the whole 225 illigitimate pregnancies at the S.M.M.P. therefore, 10.22 per cent were abortions. These figures may be vitiated by the fact that on the whole less abortions necessitate hospital admission than term deliveries, especially in this class of patients. The incidence of congenital malformations cannot also be compared in the 2 groups, for one group is scrutinised by anatomical dissection, whereas the other is only subject to external inspection.

PART 3

PATHOLOGICAL ASPECTS

The Foetus
Pathology
Congenital Abnormalities

The Umbilical Cord

The Placenta and the Membranes

I. THE FOETUS

Section A.

Pathological Classification.

The difficulty of establishing a sound classification of abortion material on a pathological basis is an old one. It has long been realised that the growing ovum might be defective, maldeveloped or otherwise pathological in such a way as to dictate or contribute to its fate in abortion, and evidence of such abnormality is often born by the abortus and is amenable to examination. Unfortunately, however, abortion specimens are very often incomplete or mis-shapen by the trauma of the abortion process or the handling of the products, whether surgical or otherwise. To the pathologist who has to receive abortion specimens as they come, the question of the presence or absence of abnormality is frequently impossible to answer, and the need for a pathological classification in which to fit the different types of material has always been pressing.

A search for a suitable classification commenced over a century ago. Granville's "Graphic Illustration of Abortions" (1834) was followed by other works including those of Panum (1860), Muller (1847), His (1882, 1891), Giacomini (1882 - 1891) and Mall (1900, 1903, 1904). In 1921, Mall and Meyer produced their

classical work, reporting the pathologic ova in the first 1000 accessions to the world famous embryologic collection of the Carnegie Institution of Washington. Their work remains the standard on the subject, and their methodology and classification remain the basis that was to be adopted by subsequent workers, with only minor modifications.

Pathologic abortuses in the original classification were those in which the embryos were absent, defective or macerated. Hertig and Sheldon (1943) argued, with good reason, against the inclusion of macerated embryos in the pathologic group for no other reason than maceration. They considered that, broadly speaking, those embryos were essentially normal but had died in utero for one reason or another.

The following is the classification of pathologic abortuses devised by Mall and Meyer, as modified by Hertig and Sheldon:

1. Pathologic ova with absent or defective embryos.
2. Embryos with localised anomalies.
3. Placental abnormalities.

The group of pathologic ova is classified in more detail as follows:

Group I. Villi only: This material contains only chorionic villi, whether normal or abnormal. Obviously, this group is one of convenience only, since it merely classifies the material submitted, which may or

may not adequately represent the relation between ovular and maternal tissues. The incomplete nature of the material is obvious.

Group II. Empty chorionic vesicle: This type of specimen when intact (as it often is) represents the most pathologic type of ovum with which the pathologist has to deal. There is no derivative of the inner cell mass, that is the portion of the fertilized ovum destined to give the embryo. If the chorion is ruptured, one might have reasonable doubt about the essential pathologic nature of the ovum; that is, the normal embryo with its surrounding amnion might have been lost during the abortion. However, if trauma has produced such an artefact in a normal ovum, one can usually see evidence of the torn stump of a normal umbilical cord with its radiating vessels.

Group III. Chorion containing empty amnion: This type of ovum is only slightly less pathologic than the previous one, there being no evidence of an embryo although the amnion is present. Members of this group are likewise, valid if intact - as they often are - although if ruptured, erstwhile normal ova with the embryos missing can usually be detected and differentiated

from true Group III specimens.

Group IV. Chorion and amnion containing Nodular Embryo: This type is truly pathologic, as the embryonic mass consists merely of a disorganized group of embryonic cells. Artefacts in this group would consist of the macerated remains of an otherwise normal umbilical cord within either a ruptured or intact amnion.

Group V. Chorion and amnion containing Cylindric Embryo: If the head end of the embryo can be recognized even though it does not possess any other features of an embryo, such a specimen is valid for this group.

Group VI. Chorion and amnion containing Stunted Embryo: It is possible to recognize the embryonic form, although it is much smaller than it should be for the menstrual age of the specimen. In addition, one or more portions of the embryo are atrophic, deformed or degenerated. These embryos are usually not macerated. This is a valid group whether the chorion and amnion are ruptured or not, since the embryo has to be recognized before the specimen may be placed in this category.

In his version of the classification, Javert (1957) excluded the secundines, and coined the term "ovofoetus" to indicate that part of the fertilized ovum destined to be the foetus, in its various stages of development. He also recognized the three stages of development that His (1880) had divided gestation into.

These stages are:

1. Ovum: From fertilization up to 2.9 weeks.
2. Embryo: From 3 to 5.9 weeks.
3. Foetus: From 6 weeks onwards.

Javert therefore classifies the pathology of the ovofoetus into the following categories hereby summarised:

Group I: Pathologic ovum (empty sac)

Group II: Defective embryo (agenesis, amorphous cylindric, nodular and stunted)

Group III: Abnormal foetus (congenital abnormalities, but not merely maceration)

Planning on the same basis, I have adopted the following pathological classification of abortion material which seems to be both workable and reasonable:

A. Specimens of no conclusive diagnostic value: This group includes curettings, fragments of placental tissue, ruptured empty sacs, most carneous moles and placentae without a foetus.

B. Pathological ova in the form of an intact sac without any trace of a foetus inside. (Figs. 1 and 2)

C. Abnormal embryo: Nodular, cylindric and stunted. (Figs. 3 to 7).

D. Abnormal foetus whether fresh or macerated.

Findings.

In this series the number of specimens of no

conclusive value in the diagnosis of normality or abnormality of the ovofoetus was 98. Of these 75 were fragments of tissue produced spontaneously or by evacuation, 9 were ruptured empty sacs, 1 was a carneous mole with no distinctive foetus and 13 were placentae without a foetus.

There were 16 instances of pathological ova in the form of an intact sac containing no embryo. In all these specimens the umbilical cord also was missing. There were, however, 14 other specimens where the embryo was present but the cord was absent; these will be discussed later on in the section on the umbilical cord. None of the specimens contained a cord.

Twenty-five embryos were contained in 23 specimens including 2 cases of twins. Only 5 embryos were assessed normal, 3 in an intact sac including 1 termination, 1 of twins born in a ruptured sac and 1 embryo found free amidst fragments of tissue and clot. There were 13 nodular embryos in all. Six of these were in an intact sac, of which 2 had no umbilical cord. Seven nodular embryos were found in a ruptured sac, including one in each of 2 sets of twins. Three had no umbilical cord including those 2 twins. Three cylindric embryos were found: one in an open sac, one a twin in an open sac with its own cord represented with a small cyst and its co-twin a nodular embryo with no cord, and the third a dismembered embryo where little more than a

cephalic end was found free amidst blood clot and tissue fragments. Stunted embryos counted 4. One was found in an intact sac, and under the dissecting microscope both its upper limbs were club ended without finger processes. Two were contained in intact sacs in a case of a haemorrhagic mole. The fourth was contained in a ruptured sac. In 3 cases (1 normal in intact sac, 1 nodular in open sac and 1 stunted in open sac) a little white seedling of tissue about 2mms. in diameter protruded into the cavity of the sac about 1 cm. away from the embryo, it showed no structure at all and it was not possible to conclude whether it represented an early arrested twin or any other speculation, even when expert embryological opinion was sought (the shadow or gossamer form of Mall and Meyer).

The group of foetuses counted 168 from 164 abortions including 4 sets of twins. In 87 foetuses there was maceration to varying degrees, an incidence of maceration of 51.79 per cent. In spite of maceration autopsy was attempted in every case, but the completeness of the autopsy was certainly affected by the amount of maceration and tissue autolysis and was often very advanced. The term NAD (no abnormality detected) was therefore preferred to "Normal" in concluding such autopsies, with a comment on the degree of maceration which sometimes amounted to: "viscera: macerated beyond autopsy". Brain tissue was notable in its relatively

early liquefaction, whereas pulmonary and cardiovascular configuration were well preserved in quite macerated foetuses. Gross external - or internal - abnormalities were easy to spot, but a systematic dissection was carried out in search of deviation from the standard normal, no matter whether it was thought to be lethal or completely harmless. Sixty-nine foetuses from 67 cases including 3 cases of twins showed what was considered to be deviation from the normal, and they will be detailed later in the section dealing with congenital abnormalities.

Table XII represents the incidence of various groups of material received in relation to pathology of the ovofoetus. Table XIII shows the incidence of normal and abnormal ovofoetus by stage of development.

Discussion.

The incidence of ovofoetus in this series was 50.24 per cent, whereas it was 31 per cent in Mall and Meyer's series (1921), 52 per cent in that of Hertig and Sheldon (1943) and 34.9 per cent in that of Javert (1957). The first authors included macerated foetuses amongst the pathological group. This was not followed by the authors of the last two series, who found an incidence of maceration of 24 and 41.6 per cent respectively, compared to 51.79 per cent in this series.

It will be noticed from Table XIII that of the

Specimen		No.	% of total
Material of no conclusive diagnostic value			
Fragments and curettings	75		
Ruptured empty sac	9	98	31.92
Carneous mole	1		
Placenta only	13		
Ovofoetus			
Pathologic ovum (empty sac)		16	5.21
Embryo			
Normal		5	1.63
Abnormal			
Nodular	13		
Cylindric	3	20	6.51
Stunted	4		
Foetus			
N A D		99	32.25
Abnormal		69	22.48
TOTAL		307	100

TABLE XII. Classification of our abortion material in relation to pathology of the ovofoetus.

N.B. Twins are counted separately.

Stage	Total		Normal		Abnormal	
	No.	%	No.	%	No.	%
Group I Ovum	16	7.66			16	100
Group II Embryo	25	11.96	5	20	20	80
Group III Foetus	168	80.38	99	58.93	69	41.07
TOTAL	209	100	104	49.76	105	50.2

TABLE XIII. Incidence of normal and abnormal ovofoetus according to the stage of development.

group of ova 100 per cent was pathological, whereas such incidence was 80 per cent for embryos and 41.1 per cent for foetuses. The diminishing incidence becomes even more manifest as pregnancy continues beyond 28 weeks and ends in premature labour, and the incidence reaches its minimum with full term foetuses. This would denote a progressive gain in fitness as the conceptus goes through the oval, embryonal and foetal stages, in accordance with the rule of survival of the fittest.

Speculations into the factors underlying ovo-foetal pathology are age old. At the Carnegie Embryological Laboratory, which has been studying the subject for a long time, one hypothesis gave way to another over the years with the progress of human embryology and the related branches of science (Corner, 1944). Mall (1908) postulated that environmental factors in the form of endometrial disease were responsible, a view that gained support by experimental work producing abnormalities by placing amphibian and fish embryos in abnormal environments. Soon afterwards the cyclical menstrual histological changes of the endometrium were elucidated by Hitschmann and Adler (1908), and what had been known to Mall as "glandular endometritis" proved to be a normal pattern. In the light of this new knowledge of the mammalian cycle, Mall's views were challenged, because observations in animals showed that individual embryos frequently developed abnormally or died in the midst of

their normal littermates in the same (normal) uterine environment (Corner, 1923). This laid the foundation to the concept that constitutional defects intrinsic in the fertilized ovum itself existed and could be responsible. In 1949 Hertig and Rock reported on a series of 36 early human ova, recovered from hysterctomy specimens of 166 fertile potentially pregnant females operated for various gynaecological reasons. In 40 per cent of these ova they found an abnormality of some degree or another. Knowing that the previous abortion incidence of those mothers was 12 per cent, they postulated that about 40 per cent of human ova were defective to some extent, but that only 12 per cent would be sufficiently bad to result in clinical miscarriage.

Huber, Melin and Velios found the conceptus to be abnormal in more than half of the women who abort, and that if it were possible to prevent these abortions, the incidence of such abnormalities as hydatidiform mole would have increased manyfold.

The shift of emphasis from unfavourable environment to intrinsic ovular defects as the responsible factor did not, however, furnish the final answer. There is little doubt at present that both factors can operate in producing prenatal pathology. Moreover, ovular defects may be primary or secondary to such environmental causes as anoxia, radiation, pyrexia or

disease (Corner and Bartelmez, 1953), that can affect the fertilized ovum or even either gamete before fertilization. More recently Roth (1962) in his discussion of the "stale egg" concept in human abortion, pointed out that fertilization of the ovum relatively late after ovulation could be the underlying factor, and not necessarily a primary pathological ovum; this had been proved earlier by experiments on animals (Young, 1953).

It will be apparent, therefore, that in a great many cases, however complete the data and the material examined are, it will remain impossible to find a cause, or to incriminate one or more pathological factors that might be found, as the underlying abortive factor. Jeffcoate and Wilson (1957) suggested the onset of symptoms as a clue to aetiology: an onset with bleeding or death in utero indicated a fault foetal or placental in origin, whereas an onset marked by painful uterine contractions showed the fault to be of uterine origin. It can also be assumed that when a living foetus is aborted the cause is environmental. Javert (1957) made use of the ovofoetal classification by chronological staging for correlating possible causes, as seen in Table XIV.

Pathologic Classification		Causative Factors
Group I.	Pathologic ovum	Late impregnation and implantation; defective ovum, faulty implantation; faulty development, abdominal stalk defects; decidual haemorrhage, anoxia, conceptus inversus.
Group II.	Defective embryo	Environmental factors, decidual haemorrhage; uterine contractions; cord defects; circulatory system undeveloped, hydatidiform degeneration; anoxia.
Group III.	Abnormal foetus	Uteroplacental organ pathology, premature separation of placenta, placenta praevia, decidual haemorrhage, premature dilatation of cervix, premature rupture of membranes, cord complications, congenital anomalies, hydatidiform mole, uterine contractions, avascular villi; anaemia of foetus; anoxia hyponutrition, avitaminosis.

TABLE XIV. Causative factors in pathologic ovofoetus.
(after Javert)

Section B.

Congenital Abnormalities

Findings

As already mentioned, 168 foetuses were examined. Foetuses of very small size were inspected under the dissecting microscope and sent for histological sectioning to determine the nuclear sex. As a general rule foetuses 12 weeks or more old were suitable for anatomical dissection, but advanced maceration sometimes made this incomplete to a variable degree. The conclusion "no detectable abnormalities" was drawn in 99 foetuses, including one set of twins and one member of a set of twins.

The following abnormalities were found in 69 foetuses, including 2 sets of twins and one member of twins; they were found separately or in various combinations and they have been classified according to the systems involved.

Gastro-intestinal system: (Figs. 9 to 21).

Imperforate anus	4
Umbilical hernia or exomphalos	5
Meckel's diverticulum	2
Tumour of first part of duodenum	1
Para ileal tumour	1
Persistent mesentry of	

descending colon 7

Fenestration of lesser omentum 2

Mesentry of gall bladder 1

Persistent fissuring of the liver 1

Accessory splenic tissue 1

Volvulus of small intestine 2

Meconium obstruction with perforation in a 19 week foetus 1

Cardiovascular system

Persistent left superior vena cava - transposed aorta - pulmonary atresia - atresia of pulmonary artery - ventricular septal defect (Fig. 22) 1

Arcuate demarcation of ventricles - large ductus arteriosus (Fig. 23) 1

Small heart (2.89 gms. in relation to 9.15 gms., the weight of the heart of its almost equal sized twin. The cord of the smaller twin had a velamentous insertion, and although anastomosing, it was obviously responsible for only one-third of the combined

placenta) (Fig. 61)	1
Missed umbilical artery	9
Urinary system: (Figs. 24 to 29)	
Hydroureter	4
Hydroureter + hydropelvis (1 unilateral - 1 in one only of twins)	4
Microureters	1
Hypertrophied kidneys	1
Cyst of the urinary bladder	1
Deficient musculature of urinary bladder (transparent bladder histologically very deficient in muscle)	1
Nervous system: (Figs. 30 to 36)	
Spina bifida (one case of spina bifida occulta)	3
Encephalocele	1
Gap of the skull vault with early encephalocele	1
Fenestration of falx cerebri	2
Hydrocephalus	2
Respiratory system: (Figs. 37 and 38)	
Trilobed left lung	7
Bilobed right lung	6

Underdeveloped left lung (2.6 gms. compared to 7.8 gms. for right lung)	1
Endocrine glands:	
Suprarenal nodule (? adenoma) (Fig. 39)	1
Integumentary:	
Hare lip and cleft palate (Fig. 40)	1
Polydactyly (Fig. 41)	2
Low set ears (Fig. 43)	2
Genital system:	
Persistent cloacal membrane (phallus without urethra vulva or scrotum or anus)	
Skeletal system: (Figs. 42 to 51)	
Micrognathus	2
Tail process (ectopic pregnancy)	1
Absent lower limbs	1
Boney defect of skull	2
Asymmetrical growth of lower limbs	1
Absence of fingers or toes	4
Absent forearm (phocomelia with one finger process)	1
Various deformities of form	14
Hydrops foetalis (Fig. 52)	1

Examples of these are illustrated in Figures 9 to 52 with brief notes. In 42 foetuses one abnormality was diagnosed, whereas in the remaining 27, multiple anomalies were found. The maternal age is illustrated in Table I. The gestation age was 8 - 12 weeks in 4 cases, 12+ - 16 weeks in 22 cases, 16+ - 20 weeks in 14 cases, 20+ - 24 weeks in 19 cases and over 24 weeks in 8 cases. The pregnancy rank of the present abortion was first in 18 cases, second in 9, third in 10, fourth in 11, fifth in 9, sixth in 2, seventh in 3, ninth in 1 and unknown in 4 cases. The sex of the foetuses was male 42, female 21, with 7 indeterminate. The sex ratio was therefore 200/100.

DISCUSSION

Introduction

It is not always easy to define what is to be considered a congenital abnormality and what is not to be. As Morison (1963) points out, every animal possesses morphological features and functions which permit it to be recognized as a member of its species. The common possession of these features and an appreciation of their normal range of variation, allows the definition of an abstract normal individual, or "norm" for the species. Gross structural departure from this standard normal should be obvious and is usually associated with such disturbance of function as to make life unhealthy or impossible. Some abnormalities represent an important cause of foetal wastage from abortion, stillbirth, premature birth and neonatal death or even later death or life with a degree of disability (Ellis, 1963). It is when such structural variation from the standard is trivial or not accompanied with apparent functional disturbance that the line is less sharply drawn between anatomical variation and congenital malformation. Moreover, gross disturbance of function can occur in the absence of structural abnormality even on histological examination, as happens in many instances of inborn errors of metabolism (Garrod, 1923). The consensus of opinion is to limit the term to disturbance

of structure recognizeable with the naked eye, but even then the decision is often subjective and so influenced by personal opinion as to explain the wide range of incidence in various statistical surveys.

Since the dawn of history Man showed an acute interest in congenital abnormalities, but, until the very near past their interpretation was based entirely on demonology and superstition (Warkany, 1959). Of the earliest attempts to rationalise the occurence of congenital malformation on scientific grounds based on observation in animals was Harvey's theory of arrested embryonic development which appeared in 1651, to be almost completely ignored for the next 150 years. The 19th century brought with it great advances in the study of teratology: both descriptive and experimental. The observations of Mendel (1886) on plant hybrids drew attention to the similar behaviour of some human malformations, although those observations took 40 years to do so. Thus, genetic explanations made their appearance for the first time besides the environmental ones, revealing that the origin of a malformation may date back several generations before. One of the important studies of the subject is the work of Ballantyne published in 1904. Besides a superb historical review and full bibliography, his series of congenitally malformed foetuses is both so rich and described in such a masterly

fashion, that the book remains a standard classic that is far from being outdated.

As other causes of foetal wastage progressively diminished with the advance of preventive medicine, therapeutics and obstetrics, congenital abnormalities have been assuming increasing prominence in mortality and morbidity statistics of infancy and childhood. Much light has been focused on the subject as a public health problem, and questions of both their aetiology and morphogenesis stimulated many minds (Warkany, 1964).

Morphological Classification

From the morphological point of view, the following classification of congenital malformations is both clear and adequate (Browne, 1933; Ellis, 1963).

(1) Failures of development:

a) Complete absence of an organ or part of an organ - e.g. anencephaly or absence of digits.

b) Small size of an organ or part of an organ e.g. microcephaly.

(2) Failures of fusion: e.g. hare lip - spina bifida.

(3) Failures of differentiation: e.g. syndactyly - horseshoe kidney.

(4) Failures of atrophy: e.g. imperforate anus - Meckel's diverticulum.

(5) Failures of invagination; e.g. absence of anus or vulva.

(6) Failures of migration: e.g. malrotation of gut - ectopic glandular tissue.

(7) Failures of canalization: e.g. atresia of gut - hypospadius.

(8) Reduplication: e.g. polydactyly - double ureter.

(9) Hypertrophy: e.g. hemihypertrophy - adrenal hypertrophy with pseudohermaphroditism.

(10) Newgrowths: e.g. angioma, tumours.

(11) Aberrant development and displacement: under this heading may be included a variety of malformations in which an organ has developed in a manner which is atypical, but which does not fall into one of the above categories, e.g. cystic fibrosis of the pancreas, or where otherwise normal tissue is found in an abnormal position, e.g. gastric mucosa in a Meckel's diverticulum, or where a whole organ or part of an organ is displaced.

It is comparatively common to find various combinations of malformations, they may have a common aetiology, but need not necessarily fall in the same morphological categories.

Aetiological Aspects

It is when we come to consider the aetiological

aspects of congenital abnormalities that the picture becomes confused. Discussion of their origin has tended to become a controversy as to which of the two long known factors - genetic versus environmental - is concerned. Unfortunately the answer to such a question is often impossible. Many effects brought about by non-genetic influences are exactly the same as the genetically produced ones, although of course they will not be transmissible to subsequent generations. A look at the end result will not, therefore, determine its origin (Morison, 1963).

Fraser (1959) has shown that only a minority of congenital malformations have a major environmental cause, a minority have a major genetic cause, but that most malformations probably result from complicated interactions between genetic predispositions and subtle environmental factors. He states that there is certainly no one cause, and (almost certainly) no one cure for malformations. It may be that genetic factors render the embryo sensitive to certain minor environmental variations, and that the phenomenon is governed by what Crew (1962) calls "genetic inclination and environmental provokation."

Our current views on the effects of environmental variations are based on full awareness of the dynamic embryological sequences that the embryo is undergoing during its development (Ingalls, 1960). The embryo consists of various groups of cells growing and differen-

tiating at different rates during different periods of their development. This activity is directed by groups of cells called the "organizers", and by the chemical substances, inductors or evocators, they secrete, and is determined primarily by the genetic constitution of the individual (Morison, 1963). An adverse environmental agent will probably act selectively on the sites that are at the time most actively growing, and here lies the importance of timing. The nature of the noxious stimulus may vary, but whether it acts by physically affecting the cells or by chemically upsetting an area or organizer activity, an amount of damage will be inflicted that cannot be repaired later on, and the site of which will be the tissue that is at the time most active. This conception furnishes explanation to the mechanism by which a series of anomalies following for example maternal rubella can be related to the time of infection (Ingalls, 1950 and 1956; Swan et al., 1946). According to Streeter (1951), the period of active differentiation of the eye lies between the fifth and eighth weeks, and that of the cochlea between the seventh and the tenth: Ingalls found that in cataract after rubella the mean period of maternal infection corresponded to the foetal age of 1.17 months, and that in deafness it was 2.17 months. In the same sense, any other teratogenic agent will produce the same deformities if it happens to affect the embryo at the same stage, provided of course it is

not strong enough to affect less active tissue as well, or to kill the embryo.

Valuable as it is, this conception is not infallible and may sometimes be misleading (Gruenwald, 1958; Kalter and Warkany, 1959; Warkany, 1960). An organ can be damaged not only when its component cells are most actively growing but at a much earlier period, and the damage is manifested later on by interfering with the future formative activity. It has also been shown that different teratogenic agents sometimes have different effects (Wilson, 1959).

Although the search for environmental teratogenic factors has been long and intensive, only few agents have so far been clearly implicated as causes of congenital malformations in human beings. Amongst the earliest groups of malformations to be related to a specific environmental cause were those due to radiation, whether therapeutic (Murphy, 1947; Rugh and Grupp, 1960) or, less certainly, atomic (Plummer, 1952; Neel and Shull, 1956; United Nations, 1962). Hicks (1952) proved, by animal experiments, the high susceptibility of nervous tissue to ionizing radiation.

Maternal rubella in early pregnancy as a cause of congenital abnormalities was first pointed out by Gregg (1941). The estimated risk to the foetus varied inversely with its stage of development, and ranged from 83 per cent when infection occurred in the first month

to 61 per cent when it occurred in the fourth month: after that it declined to a range between 29 to 11 per cent (Swan 1949). Prospective studies on the effect of rubella on the foetus furnished more accurate statistics about the risk, incidence and effects, and also showed a higher incidence of abortion and stillbirth (Manson, Logan and Loy, 1960; Lundstrom, 1962).

Following rubella, other virus infections were investigated for possible teratogenic effect. Manson and her colleagues (1960) studied maternal measles, chickenpox, mumps, and poliomyelitis but found no evidence of such effect. Coffey and Jessop (1959) produced evidence of increased incidence of malformations to more than double that in a control group, in mothers suffering from influenza early in pregnancy, although Campbell (1953) found no influence.

Toxoplasmosis was found to affect the foetus (Feldman, 1958), producing a disease condition relatively late in foetal life. It does not produce major developmental defects, and such changes as microphthalmia and microcephaly are the result of disturbances during the growth phase and not from defective formation of organs at an earlier stage (Morison, 1963).

Another example of the injurious effect of an unsuitable environment is the unsatisfactory implantation of the foetus as occurs in ectopic pregnancy (Mall, 1908; Suter and Wichser, 1948) and placenta praevia (Record,

1956). This might partly explain the higher incidence of malformation with advancing maternal age, although other effects of age on the gamete are probably operative. Another aspect of the unsatisfactory implantation with disturbed foetal nutrition relates to the various reports of increased malformation rate following threatened abortion or antepartum haemorrhage (Landtman, 1948; Turnbull and Walker, 1956), although the evidence is certainly not conclusive. The occurrence of malformations in one only of identical twins, may thus be due to the foetus being unfavourably situated and therefore fails to develop a proper placental blood supply, and depends on anastomosis and on the heart of its twin for nutrition (Loeschcke, 1948; Gruenwald and Mayberger, 1960).

The thalidomide tragedy caused such a stir in the medical as well as in the lay press. This drug was first synthesized in Germany in 1954, and was made available there without prescription in 1957, to be used as sedative and anti-emetic. In Britain it became available under prescription in 1959 as "Distaval", produced by the 'Distillers Company (Biochemicals) Ltd.', (Lewis, 1964). Following its use an increased incidence of hypoplastic deformities of the limbs was noticed (Wiedemann, 1961); the drug was suspected to be the cause (McBride, 1961; Lenz, 1962) and it was withdrawn (Hayman, 1961). Further reports of limb deformities

followed from Germany (Pfeiffer and Kosenow, 1962; Pliess, 1962), England (Russel and McKichan, 1962; Stabler, 1962), Scotland (Spiers, 1962), Ireland (Devitt and Kenny, 1962) and from other centres. The drug probably acts by its antagonism to glutamic acid. The main effects of the drug are on the limbs, which may be entirely absent (amelia), missing the proximal part (phocomelia) or the distal part (hemimelia), as well as causing other abnormalities as talipes, anomalies of the shoulder and pelvic girdles, dislocation of the hip, syndactyly, eye and external ear anomalies, cardiovascular abnormalites, atresia of the gut and imperforate anus, cardiac, genito-urinary, abnormal lobulation of the lung and liver, anomaly of the gall bladder and appendix, bicornuate uterus, vaginal atresia and capillary haemangioma of the face (Lewis, 1964). There is reason to suspect a causative relation to the missing of an umbilical artery (Kajil et al., 1963). A correlation between the type of lesion produced and the time in pregnancy when the drug was given was shown to exist (Woollam, 1962). Just as the use of the drug was manifested by a rising incidence of limb malformation, its withdrawal was followed by a decline of the abnormality to the previous incidence (Mildenstein et al., 1964). Although the drug is now no more, it has left a living tragedy in the form of scores of living, intelligent, severely deformed growing children (Lancet,

1962). It also taught us a lesson which we shall never forget, about the possible effects of drugs on the human embryo. Another result was the setting of a panel of experts by the Ministry of Health in this country to guarantee thorough investigation before a new drug would be released (Lancet, 1963).

It was inevitable, after the thalidomide tragedy, that many - actually any - other drugs would be suspected. Phenmetrazine ("Preludin") was suspected because of 2 cases of diaphragmatic hernia following its use in early pregnancy (Powell and Johnstone, 1962). The musculinizing action of oral progestins on a female foetus is another example (Wilkins, 1960). Meclozine was banned in Sweden upon the advice of the National Board of Health, although British Drug Houses Ltd. stated that animal tests and extensive clinical trials proved the drug to be safe (1962, Lancet, 2, 1177 - 78). At the present time, conflicting views on a great many drugs keep appearing in medical journals, a situation that is likely to continue for some time to come.

Other possible environmental factors have been described with evidence that varies in strength. Examples of these are the hormonal factor in pre-clinical diabetes and hypothyroidism (Hoet, 1959), the influence of life in high altitudes possibly by causing hypoxia (Alzamora, 1953) and various other variables.

A tremendous amount of experimental work has now accumulated and still goes on, probing the aetiological aspects of malformations (e.g. Klater and Warkany, 1959: Wilson, 1959; Ciba Foundation Symposium, 1960). A treasure of interesting information has been collected, but unfortunately it cannot be directly translated to terms of human behaviour. The value of experimental teratology to the human teratologist, as well as its limitations, have been discussed by Warkany (1964).

Some deformities such as varus and valgus deformities of the limbs or asymmetry of the head have been suggested to be caused by intrauterine moulding strains, so that they are produced by pressure against the uterine wall or a uterine tumour, the pelvis, or some other part of the foetus or a twin, and are not the result of a developmental fault (Ballantyne, 1904; Parmelee, 1931; Walker, 1961). This explanation should be accepted only late in pregnancy when the amniotic fluid is diminished in volume relative to the size of the foetus (Morison, 1963). In earlier foetuses, however, there is more reason to consider the deformity as developmental, although of course oligohydramnios due to leakage or resorption of liquor cannot be always excluded. Other malformations are more appropriately considered as incidents of foetal disease rather than faulty development; examples of this category are hydrops

foetalis and intestinal volvulus.

The sex incidence of malformations is interesting. In a big group of living malformed babies McKeown and Record (1960) found that the sex ratio was 106.5 males / 100 females; which is the general secondary sex ratio to be found at birth. It is generally known that in the antenatal period male wastage is much more predominent (see Part 4 of this study). In this series this was even more exaggerated in malformed foetuses, in conformity with the opinion that the male sex is generally the more fragile one. When considering individual malformations, however, some of them showed definite sex preference, so hydrocephalus was commoner in males and anencephaly in females. These and other malformations were adequately discussed by the above authors, and they give a strong evidence to a genetic factor.

Congenital malformations with <u>specific genetic cause</u> have been discussed by Fraser (1954). Many factors, however, make their assessment quite difficult. First is the close interaction between environmental and genetic influences. Second, both genetic and environmental factors may give exactly the same anomalies. Again, different anomalies may be the expression of a common defect. Abnormalities due to dominant genes can be easily identified by tracing them back from affected

child to affected parent for many generations, except where they arise by fresh mutation. When the gene shows reduced penetrance (as in some carriers showing no clinical abnormality), the situation is more difficult (Snyder and David, 1953). Malformations due to rare "recessive" genes are still more difficult to identify, for the parents and collateral relatives are almost always unaffected, and the patients themselves usually do not reproduce the anomaly in their siblings, having only a chance of one in four with the present tendency to a small family in modern society. Consanguineous parentage may sometimes - but not always - provide a clue (Fraser, 1952; Stevenson, 1960). Types of malformation with a clear-cut genetic basis are individually rare, and collectively account for less than 10 per cent of the malformation load (United Nations, 1958).

In the great majority of malformations, however, no specific causal factor can be demonstrated. In spite of this deficit in our knowledge, various statistical correlations have been piled up for certain of them, that are significant in themselves and as a guide to further approach. Examples of these are:

1. Environmental correlation - The incidence of certain defects was found to have a relation to such variables as maternal age (Courie and Slater, 1963), season of birth (Pleydell, 1960; GuthKelch, 1962),

geographical prevalence (Penrose 1957) and social class (Edwards, 1958).

2. Familial tendency - Whereas some malformations e.g. tracheo-oesophageal fistula almost never occur more than once in a family, others tend to recur in sibships (Lamy et al., 1957), while others tend to be more frequent in the relatives than in the general population, e.g. club foot and hare lip (Book, 1948; Fogh Anderson, 1942). This might point out a genetic tendency or a familial environmental factor or a mixture of both.
3. Predeliction of a certain anomaly to a certain sex (see before).
4. Experiments in animals suggest that some "sporadic" anomalies are genetically induced.
5. Correlation with epidemics of a suspect infectious agent (c.f. rubella).

These and similar observations are not always conclusive, and are the basis for the 'epidemiological approach' to study congenital malformations.

Another approach is the study of pregnancy. Retrospective studies start with the malformed baby and enquire in a retrograde fashion. This was the method that disclosed the effects of rubella and those of thalidomide. Prospective studies start with a group of pregnant mothers and keep watching them. They are necessary to establish the exact frequency of a malformation

following exposure to a teratogen. This was how foetal risk after rubella in the first trimester came to be estimated as 5.30 per cent (Hill et al., 1958) instead of close to 100 per cent as retrospective studies made some to believe.

A study of the embryo itself is fundamental. A thorough knowledge of the normal at its various stages is the basis for knowing the abnormal. The belief that pathology is only morbid anatomy is dying slowly (Morison, 1963), and the present time witnesses a more dynamic approach to the study of the foetus, both human and experimental. Functional, physiological, pathological, biochemical, immunological and other aspects are being studied (Fraser, 1959).

Until fairly recently the 'genetic' approach to study malformations confined itself to such methods as pedigree analysis, the contingency method of detecting a familial tendency by measuring the frequency of affected members in various groups of relatives of the probands (selected affected individuals), the observations of identical and non-identical twins, the study of the outcome of consanguineous marriages, and racial comparisons. Recent advances in cytological techniques as applied to human tissues, have made it possible to grow human cells and study human chromosomes. A new frontier was opened, soon to be followed by very important discoveries. "Part 5" of this work is entirely devoted to discuss

this topic; but perhaps it is relevant to conclude this section by the words of Sir James Paget in 1882 about congenital malformations: "We ought not to set them aside with idle thoughts or idle words about 'curiosities' or 'chances'. Not one of them is without meaning; not one that might not become the beginning of an excellent knowledge. If only we could answer the question - why is this rare? - or, being rare, why did it in this instance happen?"

(2) THE UMBILICAL CORD

Observations

The following data were derived from 174 specimens, the remainder being unsuitable usually because the cord was not available. The specimen index was therefore 65.65 per cent.

In 30 specimens of an intact gestation sac the cord was absent. The foetus was absent as well in 16 cases; in the remaining 14 cases there was a foetus, stuck to the wall of the sac in 12 cases and free in the cavity in 2.

This leaves a remainder of 134 cords. There were 17 instances where the only abnormality was "thinness" of the cord, as assessed by contrast to the size of the foetus; in some of these the cord was thread like. Constriction of the cord without abnormal torsion was found in 3 cases, in 2 cases the constriction was located at the umbilical end and in the third halfway along the cord. Torsion of the cord was found in 20 cases. In one of them the cord lacked one umbilical artery and had a velamentous insertion. Abnormal looping around the neck or the body was seen in 4 cases. In 3 other cases the stigmata of such looping were seen in the form of a groove marking the constricted organ. Two of these were on the neck and in the third there was a sulcus on the right arm; the cord itself being absent.

Abnormally long cord, measuring more than 3 times the standing height of the foetus, was diagnosed in one case only, and there was no looping, torsion, knotting or other abnormality. There were no incidents of abnormally short cord, i.e. measuring less than 1/3 the standing height of the foetus. There were also no incidents of true knots of the cord. False knots were observed on many cords, often multiple and sometimes containing marked loops of vessels. Owing to the smallness of the protrusion they sometimes caused, the attempt to record their incidence was abandoned; and any way they are not abnormalities. The presence of a haematoma in the cord was reported in 6 cases.

Oedema of the cord, as assessed by inspection, was diagnosed in 10 cases, including one set of mono-amniotic twins and one case of genuine hydrops foetalis. A cyst of the cord was seen in 6 cases. The mode of insertion of the cord into the placenta was known in 93 cases. The insertion was velamentous in 8 cases (8.06 per cent). These included 3 members of twins where the other cord was central, excentric and battle-dore respectively; one case of torsion of the cord which had only one umbilical artery, one case with haematoma of the cord, and 3 cords with no other pathology. Twenty cords had a battledore insertion (21.5 per cent) including the one whose twin had a velamentous cord. The insertion was central or nearly so in 19 cases and

excentric in 46 cases; these together constituting almost 70 per cent.

Histological examination revealed the presence of "vasa chordae" other than the main umbilical vessels in 8 cases (5.2 per cent of 134 cords). Developmental vascular anomalies were found in 13 cases. There were 3 cases where degeneration was so advanced that histological examination failed to recognise the vascular pattern, and indices therefore should be calculated against a total of 131 "knowns". There were 9 incidents of absence of one umbilical artery, an incidence of 6.87 per cent. In 3 cases it was the only congenital malformation. Three foetuses showed other congenital abnormalities: one had encephalocele and trilobed left lung, one had skeletal malformations and persistence of the descending mesocolon and one had bilateral talipes. The remaining 3 foetuses were beyond autopsy on account of size and maceration. In 4 cases dissection of the iliac vessels was carried out, showing underdevelopment of the internal iliac artery on one side with equal frequency; the anterior branch of the internal iliac which is normally continued as the umbilical artery, was small and progressively thinned out along the side of the urinary bladder to fade away as a capillary before it reached the umbilicus. Amongst the cords, there was one

incident of torsion in which the cord had a velamentous insertion.

One cord was found to contain 5 vessels; the foetus had bilateral talipes. One cord contained 4 vessels and was attached to a nodular embryo. One cord contained no vessels at all and the embryo was also nodular and very small.

Funitis was diagnosed 21 times: in the form of infiltration of the stroma, vessel walls or fœtal blood stream with polymorphonuclear cells.

Excluding degeneration, the presence of false knots and the presence of nutrient vessels, 122 cord abnormalities were found, including velamentous insertion of the cord. As 17 cords showed more than 1 abnormality, the number of pathological cords (including 'achordia') was 105 out of 174; an incidence of cord pathology of 60.35 per cent. (See Figs. 53 to 69).

Discussion

Over a long span of human history man has been interested in the umbilical cord, regarding it with an air of magic and superstition, that still lingers in the folklore of some contemporary societies. Prophecies relating to fertility, virility, legitimacy and many other things were based on observing such features of the cord as its girth, specific gravity or the taste of its blood. It was used as a protector from evil spirits,

and royal cords were sometimes treasured as a sacred relic (Buschan, 1934).

The umbilical cord is the life line of the foetus. Although it completely outlives its usefullness immediately labour is complete, the life of the foetus in utero is completely dependent on it as its vital connexion with the mother, and the foetus is virtually at the mercy of this structure. It is no wonder that it attracted scientific studies from an early time. Before discussing the pathological aspects of the cord, a brief consideration of some structural aspects will be given.

Structural Considerations

Spivack (1946) studied the anatomy of the cord, reviewing the literature and adding some of her own observations. She pointed out the differences between the umbilical vessels and other vessels of similar calibre; these were: (1) the presence of folds and bead-like dilatations (the nodules of Hoboken) in the arteries, and of semilunar folds in the vein; (2) the absence of true valves; (3) the peculiar distribution of elastic tissue not as a well developed internal elastic lamina but in the form of specks, clumps or fine wavy fibrils within the inner portion of the media; (4) the strongly developed arterial media, the powerful contractions of which are ascribed by some authors to

the spiral or snail-like course of the muscle fibres; (5) the absence of adventitia; (6) the very delicate connective tissue stroma which contributes to the sponginess of the vein.

The protective role of Wharton's jelly as a buffering medium containing the vessels was discussed by de Snoo (1932). It protects the vessels from outside pressures and, helped by the blood pressure in the umbilical vessels, helps to resist kinking or deformity, and prevents compression of the vessels against each other even in the face of considerable torsion. Many authorities believe that it is a functional substitute for the adventitia as a supportive structure (Zawisch, 1955; Patzelt, 1956), so that its deficiency or degeneration may cause the vessels to be compressed or, rarely, to rupture, threatening the life of the foetus (Bergman et al., 1961).

Many authors maintain that the umbilical cord contains no nutrient vessels (Bacsich and Riddel, 1945). Javert (1957) believes that the cord derives its nourishment from the surrounding liquor, and wonders about nature's enigma in depriving this lifeline from a circulation to nourish it. Mino (1892) described nutrient vessels to the jelly but only in the region of the umbilicus. Spivack (1946), whose material was taken at least 2 inches away from the umbilicus, found vasa vasora in 3 out of 35 cords, and as already mentioned they were

seen in 8 of our cases. Young and Martin (1963) ascribed the control of calibre of the umbilical vessels to the chemical composition of the blood in the umbilical vasa vasora, assuming the complete lack of any nerve supply. The question of a nerve supply to the cord is still awaiting a final answer, since some workers produced evidence of nervous elements in the cord and placenta (Berge, 1962).

The length of the cord was found to be approximately equal to the standing length of the foetus; and this generalization holds at term (Whittaker, 1870) and in earlier pregnancy (Javert, 1957). A cord less than one third of the standing length of the foetus is considered by Javert to be abnormally short, whereas a cord three times the standing length of the foetus is abnormally long. This abnormal length has been correlated to the frequency of looping and coiling of the cord (Cuthbert, 1874; Javert, 1957; Bursztein, 1962).

The normal spiralling of the cord is due to the spiral course of the vessels within it, and was noticed at least as early as 1521 by Berengarius. Edmonds (1954) clarified the fact that the vessels are wound as cylindrical helices rather than as spirals for they remain equidistant from the central axis. The cause of the spiral twist of the cord was first believed to be inherent in the cord itself (Minot, 1892; Allen, 1892),

but later authors considered it to be due to active or passive movement of the embryo (Edmonds, 1954). Spiralling helps to accommodate a longer length of vessels in the cord; another means of which is the folding of the vessels causing the surface modulations known as false knots. Whether some control of the blood flow and velocity is also thus achieved is not yet clear. The direction of the spiralling is usually from right to left but may be variable even in the same cord. In the opinion of Edmonds it is determined by the position of the embryo in relation to the effective axis of torsion of the early gravid uterus.

Pathological Considerations.

Cord complications are of grave import to the foetus. Hasley and Douglas (1957) estimated them to constitute 21.4 per cent of causes of foetal death at term, and to give a perinatal mortality of 28.2 per cent in a series of Caesarean sections performed for fœtal distress. In earlier pregnancy, Javert and Barton (1952) pointed out their major role in the aetiology of spontaneous abortion. The cord pathology is either of congenital or acquired origin, although mixed complications often occur. Examples of the former are absent or rudimentary cord, abnormally short or abnormally long cord, coarctation (constriction) and vascular anomalies. Examples of the latter are torsion, knotting,

looping around the neck or other organs, prolapse, haemorrhage and other abnormalities.

Absence of the cord, "achordia", is due to failure of development of the abdominal body stalk. Another presumptive factor is abnormal implantation or what Javert (1957) calls 'conceptus inversus.' This abnormality is frequently associated with the pathological ovum of the empty sac variety. The foetus, however, is sometimes present, either stuck to the wall of the sac or free in its cavity. The condition is almost invariably incompatible with continuance of life, although Browne (1925) in his comprehensive review of cord abnormalities quoted a case reported by Stute where a live child was delivered at term, with the placenta directly attached over an area of 4 cm^2 of the foetal abdomen. He mentioned another case where the child was stillborn at 8 months, macerated and having hydrocephalus and exomphalos.

Constriction of the cord, in combination with or independent of torsion, was described a long time ago (Ruysch, 1691; Burdach, 1758). It is more often found in the foetal end of the cord, but might occur anywhere or near the placenta. A localized fibrotic stricture is found in Wharton's jelly, which Browne (1925) thought was only secondary to umbilical arteritis. The constriction may be lethal in itself as well as by

predisposing to torsion, and cases of "torsion-constriction" are on record where the cord was twisted off and the foetus was completely detached (Gallagher and Malone, 1956). Edmonds (1954) claimed that the lesion occurred after the death of the foetus, caused by the maceration that commenced in the foetal end in which the oxygenation from the placenta first ceased. This view was challenged by Weber (1963) for the following reasons:

(1) the constriction may be seen in the placental end; (2) deliveries of living infants have been recorded in cases of constriction; (3) most cases of maceration of the cord are not accompanied by constriction. However, the torsion may presumably continue after foetal death as a result of the uterine contractions continually altering the position of the dead foetus. Thrombosis of the vessels that is often seen in the constricted area indicates that the circulation has been reduced before the lethal result. Weber suspected a possible congenital malformation of Whartons jelly, or that it had disappeared as a consequence of degeneration as described by Bergman et al. (1961).

Torsion of the cord to the point of fatally interfering with the blood supply may occur with or without a predisposing constriction. The argument that it is a postmortem occurrence has already been alluded

to in discussing constriction. This view was favoured by Schauta (1881) on account of lack of evidence of passive congestion or infarction in the affected foetus; and other contemporary authors still adopt this opinion (Piraux, 1958). Javert (1957) opposed this view on account of:

(1) It is often the only cause found to explain foetal death; (2) simultaneous arterial and venous occlusion would cause immediate foetal death with no picture of passive congestion or infarction; (3) clinically the patient feels quickening, exaggerated foetal movement and then great stillness; to be followed by the known course of a missed abortion; (4) many cases of missed abortion have cord complications; (5) the free swimming and spinning of the foetus in utero offer an adequate explanation on an antemortem basis; (6) cord entanglements in monoamniotic twins had lead to intra-uterine death (antepartum); (7) grooving of soft parts e.g. the neck indicates an antemortem development.

Browne (1925) held the opinion that the cause was an exaggeration of the factors causing normal spiralling, especially in the presence of constriction or sparsity of Wharton's jelly. He mentioned opinions of other authors relating to multiparity (due to uterine laxity), a fall, and the effect of arterial pulsations in the presence of a poor Wharton's jelly. The possible relation to an abnormally long cord has already been

referred to. Wirtinger (1941) believed the major cause to be due to the longitudinal growth of the umbilical arteries along with the increased intravascular pressure caused by the pumping of the foetal heart giving rise to screw-like deformities or torsion of the cord. Speck and Palmer (1961) believed the cause to be the twisting brought about by the movement of the foetus in utero, working on top of an anomalous deficiency of Wharton's jelly.

Looping of the cord is a physiological condition influenced by swimming of the foetus, but is apt to become pathological if the loop becomes tight, if the number of loops is increased, if accompanied by knots or if the head or an extremity pass through the loop (Javert, 1957). It is believed that excessive length of the cord predisposes to the condition (Bergman et al., 1961). If it occurs early around the neck or a limb, a deep sulcus is formed by interfering with growth in girth at the constricted area (Fig. 58). Eventually the circulation in the cord is interfered with, leading to death of the foetus, but there are records of incidents of amputation of a strangled limb and continuation of foetal growth till term (Browne, 1925).

Cysts of the cord may arise from the allantois (Haas, 1906), the umbilical vesicle, inclusion of amniotic epithelium or, usually, from degeneration of

Wharton's jelly (Browne, 1925). Javert (1957) had one case only in his series (0.18 per cent). It may be associated with other pathology, or may itself endanger the foetus by pressing the umbilical vein.

Knots of the cord are predisposed to by an actively moving foetus in a large liquor volume (hydramnios) especially if the cord is long, but not necessarily as the loop may have occurred early in pregnancy. Loops may occur during labour and the foetus is unharmed or shows signs of distress to indicate surgical salvage. In such cases they hardly leave any mark on the cord other than the loop itself. But in loops drawn sufficiently tight to obstruct the circulation, there is permanent grooving of the cord at its site with almost complete disappearance of the jelly, the vessels are narrowed at the site, and the placental proximal side of the cord may be oedematous in relation to the thin foetal side because the circulation in the umbilical vein stops first. Browne (1925) described experimental evidence of Lefour and Oui demonstrating the effect of even a slack knot in reducing the circulation. It is the consensus however, that compared to prolapse of the cord, knots rarely cause death, because the umbilical blood pressure resists its tightening (Morison, 1963); the cord of a dead foetus very readily becomes tightly knotted.

Haematomata of the cord have been ascribed to other pathological causes than the mere trauma of tough handling by the obstetrician. Rupture of the vessels in the cord has been described, both with aneurysmal-like formation (Silbernagel and Fiddler, 1942) and without aneurysmal-like formation (Ruther, 1939). Love and Bucklin (1958) described rupture of both arteries. The aetiology is not certain, but various predisposing causes were suggested such as furcate or velamentous insertion, inflammation or inherent weakness in the vessel walls.

The thin cord has been discussed by Hall (1961), who described a "thin-cord syndrome." Inadequacy in the amount of Wharton's jelly results in what he called the "unprotected" cord. Such a cord will be less resistant to compressional or torsional forces. All except one case of abnormal torsion of the cord in this series had a thin cord. On the other hand there were 17 cases of thin cord without abnormal torsion. In abortion material the element of postmortem fluid resorption resulting in thinning of the cord cannot be denied. However, these cases of thin cord contrast markedly against cords in other cases with equal or even more maceration, and it is quite justifiable to consider that in some cases the cord is too small for the baby,

and that this might play an aetiological role in abortion.

The genesis of velamentous insertion of the cord was discussed by Waidl (1960) who critically reviewed the theories of aetiology. Under normal conditions, the decidua basalis offers the most suitable site for placental formation on account of its generous blood supply. The chorionic villi burrowing into the decidua basalis, therefore, grow and form the chorion frondosum which is to form the foetal part of the placenta, whereas the rest of the chorion (chorion loeve) atrophies. Normally, implantation of the blastocyst occurs in such a way that the abdominal stalk, the anlage of the umbilical cord, will be inserted into the chorionic plate in apposition to the decidua basalis (conceptus basalis), and the future umbilical cord will be attached to the future placenta somewhere about its centre. If, however, implantation occurs so that the abdominal stalk is inserted towards the lower pole (conceptus capsularis), then the umbilical vessels will have to travel between the membranes to reach the basalis. This mechanism seems to account for the various modes of insertion, from the central insertion to the velamentous type, in the extreme form of which the umbilical vessels traverse the lower pole of the gestation sac, forming vasa praevia. Apart from the risk of foetal exanguination from rupture of these vessels, Thomas (1963)

observed an increased incidence of velamentous insertion in cases of foetal malformation, and in cases of single umbilical artery.

The incidence of velamentous insertion in term placentae is 1.25 per cent, and that of battledore insertion is 7 per cent (Williams, 1931). The much higher incidence in abortion, especially in relation to velamentous insertion, is significant, and may have an aetiological role possibly due to inadequacy of the foetal vascular arrangement.

Developmental vascular abnormalities of the cord had no more than a brief mention in Browne's (1925) detailed review. Under the heading "A rare abnormality of the vessels of the cord" he described a section of a cord that had but one artery, and instead of a vein there were numerous small vessels, some of them ruptured. The foetus had been born at 6 months and died in a few hours, with negative post-mortem findings. Abnormalities in the vessels in the form of absence or abnormal number due to joining or division of vessels have also been described (Thomas, 1963).

The presence of a single umbilical artery, though noted many years ago, has recently aroused fresh interest. The earliest case to be reported is attributed to Vesalius in the sixteenth century. Sporadic reports of the condition followed (Otto, 1830;

Hyrtl, 1870). Hyrtl found it in 8 placentae including a set of twins and another of triplets, and noticed a male preponderance of the condition and a female preponderance in the association with congenital abnormalities, both findings not supported by subsequent series. Schatz (1900) noticed the frequency of the abnormality in acardiac twins. The condition, however, remained of anatomical interest until the appearance of the reports of Benirschke and Brown (1955) and those that followed them. They pointed out the frequent association with twins and with congenital malformations, and speculated on a possible teratogenic role or a common teratogenic factor. Thomas and Schweigert (1956) suggested that the lesion might be a cause of placental insufficiency.

To diagnose the condition the cord must be examined near its foetal end, as the two arteries may fuse near the placental surface (Hyrtl, 1870; Little, 1961). The incidence of the condition varied around 1 per cent in several series at or near term (Little, 1961; Lyon, 1960), but in twins it was as high as 7 per cent (Benirschke and Bourne, 1960). In abortion series an incidence of 1 per cent was given by Javert and Barton (1952) and Javert (1957). In this series the incidence was 6.87 per cent, which suggests an aetiological relation to abortion. Major congenital abnormalities were found in about half and were often multiple and

not confined to one system, and in autopsy material the association with malformation was higher (Morison, 1963). The routine examination of the cord was therefore advised, for the discovery of the cord abnormality would help the detection of such abnormalities that could be diagnosed, or alert the doctor to watch for internal abnormalities that may manifest themselves later on (Bourne and Benirschke, 1960). Little (1961) suspected a future predisposition to adult cardiac or vascular ailments. Faierman (1960) suggested that the presence of the anomaly in the absence of a gross external abnormality indicated a 2:1 risk of an internal malformation which might later require surgical treatment. The aetiology of the condition is unfortunately still in the dark. Little (1961) found no association with maternal age, parity, toxaemia or other obstetric maternal disorder, whereas Lenoski and Medovy (1962) found an increase in the incidence of toxaemia, prematurity and late maternal age; and, based on chromosome examination, suggested the possibility of autosomal trisomy. The 17 - 18 syndrome has been reported to accompany single umbilical artery (Lewis, 1962; Uchida et al., 1962; German et al., 1962; Heinrich and Allen, 1963). Kajil and his colleagues (1963) found a single umbilical artery in 3 out of 4 cases of thalidomide embryopathy, and suggested the examination of the umbilical cord of all thalidomide babies.

Inflammatory changes of the cord, "funitis", were reported by Kaufmann (1957) to occur in 2 - 18 per cent of all pregnancies. Javert (1957) found the condition in 2 per cent of his series of spontaneous abortions, and one fourth of the inflamed cords were degenerated as well. Leucocytic infiltration is found, and may be predominantly affecting Wharton's jelly or the walls of the umbilical vessels (Eastmann and Hellman, 1961). Formerly thought to be caused by syphylitic infection, the condition has been proved to be due to nonspecific pyogenic infection, associated mainly with premature rupture of the membranes, prolonged labour and maternal infection (Sidall and Hartman, 1926). Infection may occasionally enter the foetal circulation directly from the wall of the uterus, or may enter foetal vessels traversing the cord or membranes without going first to the maternal circulation (Novak and Novak, 1958). It has also been suggested that leucocytic infiltration occurs with the beginning of maceration after foetal death, but the fact of foetal death makes such a biological process difficult to explain. In later pregnancy, Widholm et al. (1963) have correlated inflammation of the cord with foetal distress of no known aetiology.

(3) THE PLACENTA AND MEMBRANES

Naked Eye Findings

The number of complete placentae we received was 115, a specimen incidence of 34 per cent. The shape of the placenta was discoid or nearly discoid in 49 cases, oval in 20, polygonal in 25, approximately heart shaped in 7 and nearly triangular in 13. One placenta presented a rare form: on the normal placental disc a ball shaped placental mass jutted into the gestation cavity, and its summit was the site of attachment of the umbilical cord (Fig. 70). This tumour-like mass showed normal placental histology when examined microscopically.

Evidence of placenta praevia was seen in 5 cases (Figs. 71 and 72), but the possibility of more cannot be ruled out, for usually such evidence is destroyed when the ovum leaves the uterus.

Placenta circumvallata was seen in complete form in 2 cases, and in partial form in 7 more (Fig. 73). Placenta marginata was found in 5 cases (Fig. 75).

Marginal haemorrhage (the term we used in preference to rupture of the marginal sinus) was signified by an adherent clot in 11 cases.

A well formed retroplacental clot, denoting retroplacental haemorrhage accumulated beneath the placenta.

for some time prior to expulsion, was found in 9 cases (Fig. 74).

There was 1 case of vesicular mole, and 1 case of transitional mole where the vesicles could be seen by the naked eye, and where a macerated embryo was present (Fig. 76).

Evidence of placental abnormality in the form of a tough pale maternal surface with white plaques was seen 5 times (Fig. 77).

Infection as manifested by turbidity of the membranes was seen 7 times, and the impression was fortified by the smell of the specimen (Fig. 78).

The cut surface of the placenta revealed white areas of infarction in 21 cases, and red areas in 39.

There were 4 sets of twins, 3 diagnosed monozygotic.

Microscopical Findings

Histological preparations were made for 204 cases, from available placental tissue in the form of complete or incomplete placentae or fragments of placenta.

Multiple sections were usually prepared to include different areas as well as suspected pathological areas. The structural patterns during the various stages of pregnancy were observed, as well as the various

stages of degenerations and other pathological processes (Figs. 79 to 101).

Hydatidiform degeneration of the villi (microscopic mole) was found in 27 cases. Criteria for this diagnosis were hydrops of the villus stroma, absence of vascularity and hyperplasia of the trophoblast. Care should be taken not to confuse the condition with the trophoblast of early anchoring villi, or with stromal oedema (hydropic degeneration). Avascularity should not be diagnosed on the evidence of one or few villi, for the plane of sectioning might have missed the vessels. Avascularity could also be seen in other conditions than hydatidiform degeneration, namely in fibrotic villi and in association with the progressive postmortem changes that end in the typical picture of missed abortion.

Oedema of the villi amounting to hydrops was found very frequently. It was not universal, but oedematous villi were seen amongst normal villi in the great majority of cases.

Intervillous haemorrhage was found in 18 cases.

A sample of decidua was seen in 186 cases. Decidual haemorrhage was found in 169 of them, i.e. 80 per cent, as fresh haemorrhage or as a laminated clot, and sometimes it could be traced to a decidual blood vessel. Decidual degeneration was seen in 161 cases, it was variable in degree and extent, and varied from

blurring of the cells to massive fibrinous replacement. Leucocytic infiltration of the decidua was seen in all but 3 cases, but apart from mild infiltration, it was severe enough to denote an inflammatory reaction, possibly due to infection, in 121 cases or 65 per cent. In some cases the infiltration was quite heavy, and occasionally necrotic areas with leucocytic debris formed an abscess.

Rolls of the membranes were fixed and histological sections prepared from 111 cases. Polymorphonuclear infiltration suggestive of infection was found in 15 cases. Degeneration with loss of cellular structure was seen in 48 cases.

Discussion

Interest has been growing in studying the prenatal period of life, which seems to be the proper approach to further reduction of foetal wastage, now that the art of delivery and postnatal care have accomplished the best they can in this respect. The placenta and its complex functions have become the subject of many interesting studies. The placenta represents the site of close anatomical and functional interdependence of the maternal and foetal circulations, its proper functioning and growth being essentially an expression of the two systems (Morison, 1963).

The development and structure of the placenta have been exhaustively studied (Hamilton and Boyd, 1960).

So far, unfortunately, the effort done in studying the morbid anatomy and histology of the placenta, has contributed but little to unravelling the mysteries of its function. The placenta has little meaning by itself and cannot be studied apart from the mother and foetus. Variants in form may have some importance but they bear no constant relation to its function in situ. The study of the morbid histology is also limited by the facts that it can only reflect on the state of the placenta just before delivery, only tiny portions of the organ are studied, and the study can only be made after delivery when the placenta's work is finished. A foetus can die due to placental failure in spite of perfectly normal histology and the reverse is true. Nevertheless such studies are essential in spite of their limitations. Anatomical faults can often bespeak functional ones. Histological studies have made the outstanding contribution of demonstrating the changing placental structure as pregnancy advances (Figs. 79 and 80), and the consequent inference that placental function also changes (Russel, 1960; Dempsey and Wislocki, 1944). Moreover, a structural pattern for certain pathological entities has been often established. Such studies, together with histochemical, hormonal and isotope studies,

are throwing more light on placental function. Some macroscopic and microscopic features of the placenta of abortion will be reviewed.

Morphological Aspects

Weight and Size

A pattern of increase in weight and size of the placenta with the progress of pregnancy has been described (Javert, 1957; Solth, 1962). For each weight group, there seems to exist some correlation between the weight of the foetus and its placenta (Westermark, 1925; Sinclair, 1948). The significance of this ratio in erythroblastosis foetalis and other pathological conditions is appreciated, but the wide range of normal variation leaves little field to derive conclusions from this information (Hertig, 1960). Of even more doubtful value is the diameter and thickness of the placenta, for they are no index of the surface area of the mass of branching villi, and even this latter is no index of functional capacity (Morison, 1963).

Abnormalities in Form and Location

Certain anomalies in form of the placenta give rise to well defined clinical entities, with clear diagnostic and therapeutic implications. Their interpretation in terms of placental function is, however,

less well defined. The clinical significance of such conditions as succenturiate placenta, vasa praevia and placenta praevia is well established; but what these and others mean to the baby before they are clinically manifest remains uncertain.

Circumvallate placenta has been the subject of renewed interest in recent years. A classical article on its aetiology was published by Whitridge Williams in 1927, and his theoretical deductions remain valid in the greatest part. Unfortunately, however, he made the rather dogmatic statement that, from a clinical viewpoint, circumvallate placenta was merely an interesting anomaly without any clinical significance, a view against which overwhelming evidence has accumulated (Obstetrical and Gynaecological Survey, 1961). Hobbs and Price (1940) pointed out not only its role as a cause of antepartum bleeding, but emphasized also the high incidence of abortion and premature labour. They defined placenta circumvallata as an abnormal development of the placenta characterized by a restricted growth of the chorionic plate, with oblique growth of its marginal villi into the surrounding decidua vera to form an extrachorial margin of placental tissue around part or all of its circumference. The membranes which insert into the edge of the plate become reduplicated and form a fold lying on the plate and constitute a wall of varying thickness around it. If the fold is not present, the condition

is commonly spoken of as placenta marginata. Scott (1960) combined the two types under the name "placenta extrachorialis," and described the pathology, maintaining that the only histological feature was avascularity of the chorion beyond the edge of the chorionic plate. Sexton and his colleagues (1950) blamed the condition as being the chief cause of mid-trimester abortion.

Javert (1957) discussed the low implantation of the ovum, the situation which would give rise to placenta praevia if the pregnancy continued to the last trimester. In abortion material, evidence of such low position of the placenta is frequently missed, but information derived from unintentional or therapeutic abortion specimens reveals that more implantations are found in the lower uterine segment or isthmus in early pregnancy than at the time of full term or premature delivery, and obviously they are got rid of as abortions. Moreover, Javert produced evidence of a higher incidence of placenta praevia in patients who had had threatened abortion, and a higher incidence of placenta praevia in spontaneous abortion compared to a control series of therapeutic abortion. He emphasized its aetiological role in abortion, and quoted extensive literature in support of this view.

Retroplacental and Marginal Haemorrhage

Placental disturbances dominated by haemorrhage

may also occur in the absence of placenta praevia or extrachorialis. Such haemorrhage may detach and compress an otherwise normal placenta. It might occur beneath the placenta, or at its margin. Spanner's (1956) views about a marginal sinus performing the function of venous drainage of the placenta have been challenged (Javert) on the grounds that drainage occurs at the whole surface and not only the margin, and that the anatomical integrity of such a sinus is not established; the term marginal haemorrhage has thus come into use. Limited destruction of blood vessels in the decidua can result in death of the related villi only and is at least one cause of placental infarction. More extensive haemorrhage tears away or "abrupts" the placenta, and ends in abortion. The placenta may show evidence of this by bearing a retroplacental clot which often had caused depression or cupping of the placenta due to compression. If abortion follows soon after the accident, such evidence may be difficult to elicit, and even histological appearances may be negative, for the placenta was given no time to acquire pathological changes (Morison, 1963). Sometimes retroplacental bleeding is mild but diffuse, and abortion takes place without even the expected clot. The site of haemorrhage is the decidua, as shown by a thin crust of decidua between the haematoma and the villi. Javert (1957) showed that decidual haemorrhage was a pathological

entity, it was rarely seen in cases of therapeutic abortion in spite of the trauma of curettage, and could in itself constitute the mechanism of abortion, although of course, the reasons for that haemorrhage were many and variable.

Degenerations

The genesis of fibrotic degeneration of the villus stroma was the subject of a series of studies by Grey and his colleagues (1956 - 1959). They described abnormal amounts of collagen that were deposited in the chorionic villi of the majority of cases of spontaneous abortion. They also showed that there was an associated elevation of a serologic factor which agglutinated sensitized sheep's red blood cells, a serologic response that was similar to that seen in rheumatoid arthritis patients. It was therefore suggested that those histologic and serologic findings justified placing spontaneous abortion into the same ill-defined category of illnesses called "collagen diseases." The lesions were progressive, advancing to thickening and proliferation of the stromal fibres which coalesced into a collagenous plaque, which then underwent fibrinoid degeneration and eventually became hyalinized. Whereas earlier workers (Hertig and Edmonds, 1940; Mall and Meyer, 1921) considered fibrosis or collagen

deposition to be either a secondary change during the period of retention after embryonic death or a manifestation of ischaemia due to decreased villous vascularity (which pointed to early developmental abnormality of the ovum), Grey et al. considered them to be an active process, a proliferation of collagen which in turn could become the aetiological factor in a given abortion. More recently, Eckman and Carrow (1962) pointed out the difficulty of distinguishing fibrin strands and collagen fibres that can be so closely intermingled in placental tissue, and doubted if the process was really "collagen proliferation" in the true sense of the word. They also thought the pathologic changes could be primary inciting factors in some spontaneous abortions and only secondary degenerative manifestations in others.

Interest in <u>hydatidiform</u> change dates as far back as Hippocrates who ascribed the grape mole to mucoid degeneration of the villi. Aetius of Amida in the sixth century described the condition. It was first reported in Britain by Giffard in 1734. Madame Boivin, the famous Paris midwife of the early 19th Century, pointed out that the vesicles were the product of disease of the chorion. Valpau and Meckel wrote of it and Virchow revived the Hippocratic theory as to its aetiology. The writings of Marchand and Grankel late in the 19th Century are still the basis of more modern

literature. (Savage, 1957).

In aborted ova the incidence of hydatidiform changes is much higher than is generally appreciated. As Greenhill (1957) pointed out, clinicians still consider molar degeneration rare, in spite of the fact that it is common. He made a plea to examine abortions more carefully by an expert, instead of the hurried inspection that is done before throwing the abortus away. De Lee in 1913 reported the high incidence in abortions. Mall and Meyer (1921) called attention to its frequency in early ova specially those with abnormal embryo and Meyer considered that hydatidiform degeneration was the commonest of all diseases of the ovum during the earlier months of pregnancy, and suggested that the typical large vesicular mole was the end result.

The cause of hydatidiform degeneration was the subject of many investigations. Mall and Meyer (1921) incriminated endometritis as a cause, but later investigators showed it to have no aetiological role at all (Thaigy 1938; Strauch, 1938). Due to the constant disappearance of blood vessels in the villus, it was suggested that the degeneration could even begin before the villi were vascularized; but Hertig (1935) showed that the chorionic villi in man developed simultaneously with their blood vessels, hence, the process should of necessity start in villi that at least contained vascular primordia. It is disturbance or alteration of

this vascular arrangement of the villi that is generally held to be the cause of hydatidiform degeneration (Keller and Adrian, 1938), for there is failure of draining the fluid which accumulates and distends the villi, with secondary epithelial proliferation. Hertig and Edmonds (1940) studied the genesis of hydatidiform mole, based on a series of 1027 spontaneous abortions and 74 hydatidiform moles. They pointed out that hydatidiform degeneration was prone to occur at about the fifth week of pregnancy, the time when the foetal circulation should begin to function. The highest incidence was in pathologic ova because, owing to defectiveness or absence of the embryo, such failure of foetal circulation is commonest: the vascular anlagen disappeared coincidentally with the onset of hydatidiform degeneration, both processes being a function of absence or defectiveness of the circulation. They described typical stages in the evolution, from the early hydatidiform degeneration through the transitional mole to the classic fully developed stage which is the rarely encountered hydatidiform mole. In their opinion, hydatidiform degeneration is an expression of continued physiological activity of the trophoblast (absorption and/or secretion) with resultant accumulation of fluid which cannot be utilized for lack of foetal circulation. The villi of pathologic ova of early age, whose villous stroma is normally loose and whose trophoblast is normally active, are much more

prone than the nonpathologic ova which have functioning foetal circulation of some weeks duration, a relatively dense stroma and relatively inactive chorionic epithelium.

In spite of the impressive evidence lending much plausibility to this "deficient foetal circulation" theory, doubts arose whether it was generally valid (Park, 1959). Of abortuses showing absent or defective embryos, there still remain, in most series, some 30 - 60 per cent that do not show hydatidiform degeneration. This is possibly the result of simultaneous and equal damage to both the embryo and its trophoblast such that the trophoblast could no longer transmit fluid into the stroma of the villi, and it seems reasonable to assume that this should happen to two so closely related tissues. Meanwhile, the same findings can lend themselves to other explanations, for example some form of over-activity of the trophoblast that occasions excess fluid to be secreted into the stroma. With increasing dysfunction of the trophoblast, there would be a greater degree of hydrops of the villi and greater degree of avascularity (pressure atrophy), resulting in more severe damage to the embryo; and the most pronounced hydrops would be found with the most severely affected (or absent) embryos. This explanation, it is true, does not satisfactorily explain the occasional appearance of hydatidiform change as localised areas either at the margins of the chorion frondosum or in the mature placenta.

These are better accounted for in terms of local circulatory deficiencies described by Hertig and Mansell (1956), without necessity to assume that the condition must always have one cause, namely imperfect venous drainage. Local oedema in other tissues may certainly have several causes.

In their series, Hertig and Edmonds found hydatidiform changes in 66.9 per cent of the "pathologic ova", with 10.2 weeks' mean menstrual age. In "non-pathologic ova" the mean menstrual age was 15.4 weeks and hydatidiform degeneration was seen in 11.6 per cent. Their overall incidence was 40 per cent.

In our series, of the 16 blighted ova in the form of an intact sac containing no foetus, 9 cases showed evidence of hydatidiform change i.e. 56.25 per cent, and the mean gestation age was 9.7 weeks. Of our 25 embryos, 6 cases showed hydatidiform degeneration (24 per cent), all of which were amongst the abnormal (20) group (i.e. 30 per cent). This leaves a remainder of 12 cases of hydatidiform degeneration from amongst 189 histological preparations, only 3 cases were past the 16th week of gestation. Our general incidence therefore is 27 in 204 cases whose placental tissue was histologically examined, i.e. 13.23 per cent.

The relative overall scarcity of the condition in our series is, however, more apparent than real. For whereas Hertig and Edmonds found that early pathologic ova constituted

47.4 per cent of spontaneous abortion, the "pathologic ovum" constituted only 5.21 per cent of the material which we received.

Nilsson (1957) found the condition in 50 per cent of anembryonic ova, in 30 per cent of ova with embryos shorter than 0.5 cm., and in only 3 per cent of ova with embryos longer than 0.5 cm. Huber et al. (1957) clarified the diagnostic criteria, mainly avascularity, oedema and trophoblastic overactivity, and reported on the findings in 90 intact specimens, with an incidence of 20.2 per cent, highest with the earliest and most pathological ova and diminishing with rise of gestation, age and normality. Javert found the condition in 18 per cent of his cases.

Another type of degeneration that can have a significant effect on the circulation in the intervillous space, is that associated with the formation of the substance described as <u>fibrinoid</u>. Fibrinoid is supposed to be different from fibrin (Grosser, 1927; Wislocki and Bennet, 1943), but the differentiation between them is not always easy, or - for practical purposes - necessary. Very early in pregnancy it is formed from many sources, but when it acquires pathological proportions it is formed mainly by degeneration of the surface trophoblast. Various amounts of fibrinogen seep into it and become deposited as fibrin on the surface of the degenerating villi. First affecting the main villi, it later

involves the terminal or "respiratory" villi; and with deposition of much fibrin in the intervillous space, the function of transplacental transfer can be seriously interfered with.

Infarction

"Placental Infarction", as Little (1960) pointed out, has been a "pathologic waste-bucket", being loosely applied to almost any placental discoloration or deficiency. Little critically reviewed the literature and discussed the various aetiological theories. The placental pathology produced by infarction can range from the death of a large part of the placenta, which may be detached by haemorrhage from the uterine wall, to a deposition of fibrin producing ischaemia and death of a few villi: the basic feature being interference with the intervillous blood flow (Morison, 1963). Aetiological factors were assumed to be either foetal (Eden, 1897; Williams, 1900; Bartholomew, 1951) or maternal (Young, 1914). In his survey, Little (1960) excluded lesions often described as 'white infarcts' formed of large fibrin deposits (fibrin infarcts), and also those areas of laminated blood clot often described as 'red infarcts', and which are caused by sluggishness of the intervillous circulation following occlusion of maternal venous channels (Shanklin, 1959; Huber, Carter and Vellios,

1961). Based upon the classification of Zeek and Assali (1952), Little described the following types:

Type I:

Gross appearance: Varying numbers of pale usually round nodules of slightly increased density, suggestive of compressed avascular tissue.

Microscopic appearance: Normal villi found in close apposition because the intervillous space is partially collapsed. The villous capillaries contain small or normal amounts of blood.

This type is not included in true infarcts.

Type II:

Gross appearance: Early infarct characterized by black or dark-red areas of increased density extending partially or entirely through the placenta proper, and markedly contrasting with the paler surrounding placental tissue. The areas are oval or variable in shape and are confined to the cotyledonous architecture. Previous formalin fixation helps detection of early forms.

Microscopic appearance: In the early stages the intervillous space is collapsed, the villi are enlarged and in close apposition, and the villous capillaries are engorged with blood. Usually the tissue demonstrates an almost homogeneous appearance, as if the villi were glued together. The trophoblastic nuclei contain scattered or uniform evidences of

necrosis characterized by pyknosis.

In later stages of this lesion, the nuclear changes of necrosis, pyknosis and karyorhexis, are generally uniform. The cellular detail is blurred and the cells are more acidophilic. Occasionally the intervillous space is only partially collapsed. Thromboses of intervillous blood or interlacing strands of fibrin may be seen.

Occasional difficulty may be encountered in differentiating the early stages of this lesion from areas of congestion, in which there are engorged villous capillaries in the presence of a normal or partially collapsed intervillous space. The distinguishing feature is lack of necrosis in the congested areas, but there are, however, borderline cases.
(N.B.: Another pattern of villous vascular anomaly that may be confused is that in which the villous capillaries are dilated and so numerous as to almost fill the villus. The condition may represent a primary vascular anomaly. Potter (1961) referred briefly to the condition in late pregnancy and designated it "placental hypertrophy", suggesting a relation to chorio-angioma. In our material the condition was frequently seen at the edge of infarcted areas as a transition zone between infarcted and normal villi, but was also occasionally seen apart from infarction. At term the placenta with such a lesion is big relative to the foetus which is often stillborn

(Morison, 1963). Its aetiological role in abortion, however, has not been clarified. See Fig. 93).

Type III:

Gross appearance: The late acute or early subacute lesion is pink or brown, firm, usually nodular and sharply demarkated from the surrounding tissue. It may be confluent with a Type II lesion, especially when found above an organized retro placental clot.

Microscopic appearance: Villi stain poorly. More advanced necrosis with evidence of karyolysis and disintegration of cellular nuclei. Haemolysis of foetal blood is beginning, imparting the characteristic brownish colour. Polymorphonuclear leucocytes may be seen at the margins.

Type IV:

Gross appearance: The late subacute or late infarct is firm and yellow-white.

Microscopic appearance: Marked necrosis of all villous elements, total haemolysis of villous blood and almost complete obliteration of villous architecture. The villi are acidophilic, apparently hyalinizing forming "ghost villi." Frequently, these structures are surrounded by various amounts of homogeneous pink-staining material, probably fibrin.

This type is to be differentiated from fibrin deposits, which are laminated, and microscopically contain no villi except for an occasional villous entrapped

within the fibrin network.

Type V:

Gross appearance: White gritty areas.

Microscopic appearance: Similar to Type IV but there are areas of calcification or liquefaction.

Heterotopic Trophoblast

Intervillous syncytial sprouts are frequently seen in the intervillous space, being shed off the free surfaces of the villi (Fig. 94). They are very numerous at all stages of pregnancy (Boyd, 1959; Boyd and Hamilton, 1960), and give rise to the giant cell-like masses seen on histological examination. They become free in the intervillous space and some of them enter the maternal circulation via the veins draining the decidual plate of the placenta whence they may pass to the maternal lungs (Schmorl, 1893; Veit, 1905; Park, 1958; Thomas, 1961).

Decidual giant cells are another form of trophoblastic invasion (Figs. 95, 96). In spite of doubts raised by Park (1959) about their possible maternal origin, Boyd and Hamilton (1960) produced evidence and quoted evidence based on histochemical studies to show that they are of syncytial origin. Possible functions for giant cells may be the production of enzymes to soften up maternal tissues so that the

placenta might be helped to grow, or to help in establishing a line of cleavage for eventual placental separation. A more probable function is the production of hormones. The existence of these giant cells in the decidua and even the myometrium and their continued presence for some undetermined time after parturition, raises the question as to why they are immune to a homograft reaction (Woodruff, 1958), and has a bearing on the problem of chorion carcinoma as well as to other problems of pathologic interest.

Recently, Boyd and Hamilton (1964) described groups of ectopic trophoblastic material in the connective tissue cores of the villi. Using special staining techniques and resorting sometimes to serial sectioning and reconstruction, they showed that these "trophoblastic buds", as they called them, were present at all stages of pregnancy after the 20 mm. C.R. length embryonic stage, that they were connected by a stalk to the trophoblastic frame-work of the villus and that the buds possessed cytotrophoblast on their periphery surrounding a central core of syncytium. Such a reversal of the normal relationship of syncytium and trophoblast lends support to the theory that they arise by ingrowth of the investing trophoblast of the villi. In their opinion, it is these stromal trophoblastic buds that give origin to the syncytial emboli in the foetal circulation first described by Salvaggio (1960). The larger of them

might be the explanation for those developmental anomalies (e.g. intestinal atresias) that are sometimes explained by infarction of a foetal blood vessel. Whether these stromal trophoblastic buds subserve any other function beyond the probable one of producing trophoblastic hormones is not yet clear.

Wigglesworth (1962) showed that, contrary to current belief, the cytotrophoblast did not disappear after mid-pregnancy. Using special fixation and staining techniques he proved it to persist all through pregnancy, and to become hyperplastic under the stress of anoxia so that it invades the villous stroma. His findings account for the secretion of gonadotrophin that occurs after the presumed disappearance of Langhan's cytotrophoblast, and he correlates the cytotrophoblastic proliferation to various pregnancy abnormalities and their corresponding hormonal patterns.

Another structure of syncytial origin that might be found in the stroma of the villi was described by Ishizaki (1960). He maintained that calcified syncytial knots might survive the destructive effect of inflammation in foci of placentitis, to be later incorporated in the stroma of new villi. They are thus telltale marks of sites of healed placentitis, and he called them "orphan bodies".

Inflammation

Leucocytic infiltration is a very frequent finding in placental sections. It is often too dense to be passed as physiological, and on occasions there are foci of tissue destruction simulating abscess formation. The usual site is the decidua, but the reaction can also be found in the villi, in the foetal vessels and pervading intervillous and decidual haematomata from the periphery inwards. An acute inflammation is frequently associated with criminal abortion, and bacterial infection results from traumatic interruption of the tissues (Hertig, 1960). Specific infections of the placenta have been described. Tuberculosis (Schaefer, 1939), syphylis (Montgomery, 1936) and toxoplasmosis (Beckett and Flynn, 1953) affect the villous structures but may also involve the vessels of the cord. Malarial parasites have been found in the intervillous space and the associated pigment may be found in the villi, but inflammatory changes were absent (Wichramasuriya, 1935). The effects of virus diseases have only rarely been studied (Waddington, 1956).

Apart from the villous area, inflammation may affect the membranes giving the picture of chorioamnionitis. The polymorphonuclear infiltration is primarily derived from the maternal blood, but in the

later stages of inflammation and when the foetus is over 18 weeks of age there may be participation by foetal white blood cells from the chorionic plate, evidenced by a diffuse opacity of the foetal surface of the placenta (Hertig, 1960). Javert (1957) produced evidence showing that chorio-amnionitis was more frequent in cases where the membranes had ruptured long before expulsion. In some cases the inflammation affected only the membranes overlying the cervix, raising the question of the role of the infected cervix. Some cases were not preceded by premature rupture of the membranes, raising the possibility of the source of infection being some focus in the mother. Curtis (1925) claimed some success in treating cases of habitual abortion by removal of all detectable septic foci. The implications of premature dilatation of the cervix, and herniation, prolapse and premature rupture of the membranes were pointed out by Javert.

PART 4

Sex Incidence

Nuclear Sexing

On the Primary Sex Ratio

Observations

The sex of abortions was reported according to each of 3 examinations: 1. The appearance of the genitalia; 2. The histological examination of the gonads; and 3. Nuclear sexing. It is clear that information on all of these was not available in many cases, but whenever possible, all the 3 were done. Nuclear sexing studies were attempted in 277 cases. Placental tissue was used as a routine, but occasionally it was supplemented with or supplanted by foetal tissue. In 81 cases the material proved to be unsuitable for nuclear sexing on account of advanced degenerative changes. Of the remaining 196 cases, 114 were Barr negative and 82 Barr positive. There was one specimen where the findings on nuclear sexing were contradictory to the anatomical (morphological) and histological) findings. This was a female foetus, of 13 weeks gestation age, not macerated and showing no abnormalities on anatomical examination, who gave a negative result on sex-chromatin examination, with a count of 0 per cent. The foetus might have had an XO chromosome complement, but unfortunately her chromosome complement was not studied. In the overall statistics this foetus was entered as a female.

One foetus, a 16 weeks female, gave a positive count of 68 per cent, and 7 per cent of the nuclei

showed 2 Barr bodies , (Fig. 107), denoting the presence of 3 X chromosomes in at least a portion of the nuclei, but chromosomal studies were not carried out. One anatomically male foetus gave a count of 9 per cent, which is considered too high for a male. It was 12 weeks of gestation age, normal in anatomy; no chromosomal studies of it were done and it was entered in the general statistics as a male.

The count for female foetuses ranged from 27 to 68 per cent, with an average of 50 per cent. The average positivity of the count for male foetuses was 0.5 per cent, ranging from 0 - 3 per cent (this excluding the single male with a count of 11 per cent, and the female with a count of 0 per cent.)

Gonadal histology was not started from the beginning of the work but commenced later on. It became a standing routine in 248 cases, but the gonads were available (with the foetus) in only 115. Of these 115 cases, 14 were too degenerated to be diagnosed, and 101 were histologically diagnosed: 57 males and 44 females. There were no incidents of contradiction of each of the 2 gonads to the other, or of gonadal histology to macroscopic sex.

Of the different methods, nuclear sexing was the most valuable. In 65 cases the diagnosis was based on nuclear sexing alone, and could not have been possible by any other means. In only 7 cases naked eye

examination of the foetus was the only possible means for diagnosis, the tissues being too degenerated to be diagnosed by histology or by nuclear sexing. In no case was gonadal histology the only possible means of diagnosis.

Our results are shown in Tables XV and XVI.

Discussion

A. THE SEX-CHROMATIN

Barr and his co-workers (1949) noted that cell nuclei bore morphological evidence of their chromosomal sex. This was one of the blessings of the war, for the work was designated originally to study the phenomena of fatigue. Observing the histologic appearances of cats' nerve cells under various metabolic conditions, they made the unexpected discovery of a chromatin body present in the majority of nuclei in the female, but only in a small minority or none in the male. This chromatin body is now called the sex-chromatin or Barr body. Their discovery was soon confirmed and extended to the other tissues of the cat (Graham and Barr, 1952), and to other species including man (Moore et al., 1954). Realising the clinical implications of the discovery, such tests were elaborated as the buccal smear test (Moore and Barr, 1955), vaginal smear sexing (Carpentier et al., 1955), the presence of polymorphonuclear drum-sticks (Davidson and Smith, 1954), skin biopsy (Sachs and Danon, 1956) and others. Histological preparations of various tissues, tumours and cells in tissue culture have similarly been studied. The sex of the unborn foetus can be determined by examining foetal squames obtained by amniocentesis and centrifuging the amniotic fluid (Rosa and Farnard, 1951). It has been shown that

chorionic tissue shares the sex of the foetus, (Graham, 1954), and various workers have studied the nuclear sex of term placentae, placental tissue of abortions, ectopic pregnancies, vesicular moles and chorion-epitheliomata.

In human tissues, the sex-chromatin is seen in the interphase nucleus as a hyperpyknotic triangular, crescentic, square or even irregular body, sometimes bipartite, lying on the nuclear membrane and measuring on an average 0.8 X 1.2 mu. Consisting largely of desoxyribonucleic acid (D.N.A.), it stains with basic dyes and gives a positive Feulgen reaction. When present, the sex-chromatin body is readily recognized.

Having established that the nuclear sex of the foetus and that of the chorionic villi are identical, it was thus possible to sex large numbers of abortions which would otherwise be impossible to sex on account of the foetus being lost, too small or too macerated (Stevenson and McClarin, 1957; Bohle et al., 1957). In this study, placental tissue was the standard material used for nuclear sexing in the majority of cases. The sex chromatin can be seen in the stroma cells of the villi (Fig. 102), trophoblastic nuclei (Fig. 103), vascular endothelium (Fig. 104) and even in foetal erythrocytes (Fig. 105). Normally, one would have expected to obtain the best nuclei in the stroma cells, but unfortunately these are too degenerated in

many cases. Erythrocytes are usually too hyperchromatic to show the sex-chromatin. Vascular endothelial cells are too few, and many villi may be avascular. Trophoblastic nuclei have the drawback of being usually overactive, and the distribution of nuclear chromatin in blobs all over the nucleus makes sexing difficult (Stevenson, 1961); yet in many cases in this series adequate counts could be made from these nuclei.

Sex-chromatin counts were done under the oil immersion lens at a magnification of 1500, aiming at signalling 100 foetal nuclei as positive or negative. Actively mitotic, hyperchromatic, irregular, degenerated or doubtful nuclei were not included in the count. In a few cases it was not possible to find 100 suitable nuclei on account of degeneration, and lower figures had to be accepted. The lowest count for a sex-chromatin negative case was 80 nuclei (all negative), and the lowest count for a sex-chromatin positive case was 52 nuclei, of which 32 nuclei (61 per cent) showed the chromatin body.

Other factors affect the count besides experience and the quality of the material. Good staining is necessary, and on many occasions re-staining greatly clarified the picture. The sex-chromatin can be diagnosed only when seen in profile lying on the nuclear membrane, and if the nucleus happens to be rotated, the sex-chromatin will not be seen on the nuclear membrane

and the nucleus will be considered negative. It should be also remembered that a section may only include a portion of the nucleus, the sex-chromatin being in the remainder. This accounts for higher counts obtained from cell smears and full amounts of amnion, than those obtained by examining histological sections. It will be evident that the thinner the section in relation to the size of the nuclei, the more likely it is to miss the chromatin body and a lower count is thus obtained (Park, 1957).

When first discovered, the sex-chromatin was suggested by Barr and Bertram to be an index of the presence of an XX chromosome pair, and probably to represent the heterochromatin of the pair (i.e. the chromosome segments which stain atypically at some stage of their cycle.) To avoid certain difficulties (as will be shown later), terminology in terms of male and female was abandoned, giving place to the terms "chromatin-positive" for individuals whose nuclei show the high proportion of sex-chromatin characteristic of females, and "chromatin-negative" for those in whom sex-chromatin (or something like it) is present in the low proportion of nuclei characteristic of males.

In the years that followed, evidence relating to the significance of sex-chromatin gradually accumulated, which was summarized by Lennox (1960),

including the following main points: (a) No normal fertile female has ever been shown to be chromatin-negative, and no normal fertile male to be chromatin-positive. (b) It is totally uninfluenced by age (from very early embryo (Graham, 1954) to extreme old age), endocrine status, disease or any other non-genetic factor. (c) When the nuclear sex is at variance with the apparent sex, the individuals invariably show some defect of genital development which can be interpreted as a form of sex reversal. (d) In such sex-reversed individuals, the incidence of sex-linked characters such as colour blindness corresponds to the nuclear sex, and not to the apparent sex. (e) The body differs in no way except size from the mass of chromatin granules of the nucleus, and so is likely to consist, as they certainly do, of chromosomal material in its dispersed state between mitoses. (f) It can often be seen to consist of two equal halves partially separated (Lennox took this as a support to the idea of an origin from a pair of chromosomes).

Certain limitations, however, of nuclear-sexing were also realized. These were mainly species differences, technical difficulties especially with histological methods, and the difficulties arising from tissue damage and autolysis. The method can distinguish only two

categories: those with 2 or more X chromosomes and those with less than 2. No assumption can be made about the presence or absence of the Y chromosome, or its number, and therefore the method is a useful adjuvant but not a substitute for detailed chromosomal studies in cases of sex chromosome anomalies.

Since 1953, nuclear sexing has been used as a diagnostic test, and in spite of the limitations already referred to, a considerable variety of applications has proved possible.

The most important role of nuclear sexing is in the investigation of the diverse conditions grouped together under the term "Intersexes" (Lennox, 1960). Its role in the detection of individuals with such abnormal chromosomal patterns as XO (Turner's Syndrome), XXY (Klinefelter's Syndrome), XXX (multiple X) and their genetic variants, is obvious. In such conditions of ambiguous sex such as the congenital adrenogenital syndrome, it provides a rapid clue as to the real sex of the newborn infant upon which treatment is immediately carried out.

A survey carried out in Edinburgh by Dr. N. Maclean of sex chromosome abnormalities among over 20000 live births, by examining a buccal smear, lead to the discovery that among the males 2.1 per thousand had the XXY constitution and its derived mosaics, and among the females there were 1.2 per thousand XXX and 0.4 per

thousand XO (Court-Brown, 1962).

Similar surveys among the inmates of mental institutes lead to the discovery of an unexpectedly interesting bag of sex-chromosome abnormalities (Sanderson and Stewart, 1961; Davies, 1963).

It is true that nuclear sexing cannot always pinpoint the actual sex chromosome abnormality, as shown from Table XIV (after Barr and Carr, 1962). Yet on the other hand, studies of the X chromosome abnormalities by means of culture and direct chromosome examination, is incomplete unless interpreted against the background of nuclear sexing, In cytologic chromosomal preparations, the X chromosome cannot be distinguished from the autosomes of pairs 6 and 7. It follows, therefore, that it cannot be concluded that, in a woman with 47 chromosomes, an additional chromosome is an X chromosome simply on the grounds of its size and morphology. But if 2 Barr bodies can be seen in a proportion of her Buccal cells, then the additional chromosome is presumed to be an X and the woman to have an XXX sex chromosome complement. In the same way if a woman has a chromosome count of 45 and is missing a chromosome in the range in which the X is normally found, and at the same time she has no sex-chromatin bodies, then her chromosomal sex is presumed to be XO. Furthermore, variations in the size of the sex-chromatin body may be associated with morphological abnormalities of one X chromosome.

Number of Barr bodies	Sex chromosome complexes
0	XY, XO
1 (but poorly developed)	Xx
1	XX, XXY, XXYY
2	XXX, XXXY
0/2	XO/XXX
3	XXXX

TABLE XIV. (Barr and Carr, 1962).

The number of Barr bodies and the relevant sex chromosome patterns.

Deletion affecting part of an X chromosome will result in a low count with small sex-chromatin bodies, whereas, conversely, a proportion of the bodies are unusually large where the abnormality is an isochromosome formation for the long arm of the X chromosome (Jacobs et al., 1961).

Jacobs and her colleagues (1961) produced evidence that 40 per cent of cases of primary amenorrhoea either have an abnormal sex chromosome complement or are examples of sex reversals. Court Brown and MacGregor (1963) studied a group of patients with primary amenorrhoea and found that diminished height was a reliable guide to the presence of sex chromosome abnormalities, and correlated this with the count and the size of sex chromatin bodies.

Ante-Natal Sexing of the unborn foetus is another application that so far has been little used in practice. It might come into more use in relation to conditions where there is a history of a sex-linked hereditary developmental defect of an unusually serious nature (Barr and Carr, 1962), or in connection with the prophylactic aspects of Rh iso-immunization (Scott, 1963).

The sexing of abortions and its bearing on

identifying the relative foetal wastage and the primary sex ratio will be discussed in the next section

Nuclear sexing would seem to be a very obvious means of following the fate of grafts transferred from individuals of one sex to the other, and thus make possible important contributions to the study of the homotransplant problem (Davidson et al., 1958); Woodruff and Lennox, 1959; Lennox, 1960).

Nuclear sexing was used to elucidate some anatomical problems. When the foetus was male, the placental septa were proved to be of maternal origin by means of nuclear sexing, having been disputed for a long time (Klinger and Ludwig, 1957).

It has also been used in the investigation of tumours. Park (1957) confirmed that trophoblastic tumours had the same nuclear sex of the foetus, with few exceptions which he believed to be due to derivation of the tumour from the trophoblast of an earlier pregnancy. Hunter and Lennox (1954), Moore and Barr (1957) and others, showed that the majority of tumours had the nuclear sex of their host. With more actively growing tumours, anomalies such as doubled or absent sex chromatin were sometimes shown to occur

(Aitkin, 1958), but these were ascribed to chromosomal errors in dividing malignant nuclei in the form of duplication or deletion (Hienz, 1961; Obstetric Survey Editor, 16, 574, 1961). Ohno (1963) has recently discussed the behaviour of tumour cells in males and females, in the light of autosomal control of the behaviour of sex chromosome chromatin. He points out that in a normal female, tumour cells with a diploid autosomal set and an XXX complement, will still be sex-chromatin negative if, during the process of malignant transformation, the condensed X of a normal somatic cell was lost and the three X's of the tumour cell were derived from the extended X.

The origin of sex chromatin is still the subject of active research. At the beginning it was subtly described as a morphological expression of the heterochromatin found in the XX chromosome complement found in normal female nuclei (Graham and Barr, 1952). This gained support from the fact that it is found in females, with XX chromosomal complement, and absent in males, with XY. The amount of heterochromatin in the male was considered to be so small as to be submicroscopic, and therefore could not be seen except only in sporadic nuclei when it became swollen during certain

phases of metabolism. It was not found in the nuclei of females with one X chromosome only (XO : Turner's Syndrome), but was found in males with XXY complement (Klinefelter's syndrome). If more than 2 X chromosomes were present, each extra chromosome was found to produce an extra Barr body; and it was correctly assumed that the number of Barr bodies was equal to the number of X chromosomes minus one.

A simple but important question, however, remained without an answer: If it takes 2 X chromosomes in the normal female to produce the normal single Barr body, why should each single supernumerary X chromosome alone be capable of producing an extra Barr body? This question could not be answered on the basis that a single X chromosome could produce the Barr body, because the latter is absent in males, who have indeed got one X chromosome. May it be that the X chromosome of males - and that of XO females - is different from the second X of the XX normal female?

Evidence gradually accumulated that a single X chromosome was capable of producing a mass of sex chromatin, provided that certain - as yet poorly understood - requirements were fulfilled. In the spruce budworm (Smith, 1945), the silkworm (Frizzi, 1948) and the domestic chicken (Kosin and Ishizaki, 1959), there

is sex chromatin in the interphase nuclei of females, but not of males, although the female is the heterogametic sex with an XY or XO sex chromosome constitution. Grumback et al. (1960) demonstrated an XO chromosome complex in certain chromatin positive females with gonadal dysgenesis. Bahner et al. (1960) reported a case of Turner's syndrome with fully developed sexual characteristics and fertility.

In 1961 Mary Lyon proposed her hypothesis on the behaviour of X chromosomes in XX females. This followed the work of Ohno and Hauschka (1960), who showed that in female mice one chromosome of mammary carcinoma cells and of normal diploid cells of the ovary, mammary gland and liver, was heteropyknotic. They interpreted this chromosome as an X chromosome and suggested that the sex chromatin was composed of one heteropyknotic X chromosome. They left open the question whether the heteropyknosis was shown by the paternal X chromosome only, or the chromosome from either parent indifferently. Lyon suggested that the evidence of mouse genetics indicated: (1) that the heteropyknotic X chromosome could be either paternal or maternal in origin, in different cells of the same animal; (2) that it was genetically inactivated. She based the evidence on (i) that the normal phenotype of XO females in the mouse (Welshons and Russel, 1959) showed that only one active X chromosome is necessary

for normal development including sexual development; (ii) the mosaic phenotype of female mice heterozygous for some sex-linked mutants. She studied the coat colour of mice carrying sex-linked colour genes, and explained her findings on the hypothesis that "in the male the single X remains extended and active. In the female one X is extended and active, the other remains aggregated (hence the visible sex-chromatin), synthesizes its D.N.A. later than all the active chromosomes and takes little or no part in directing cellular activities." Lyon postulated that the choice as to which of two X chromosomes is to be inactivated has to be made at a definite point of foetal development, that it is a choice made at random and independently by each cell present in the foetus at that time, and that once made it is irrevocable and transmitted to all descendants of the cell. This "Lyonisation" (Lancet, ii, 1963 p. 769) occurs in man presumably about the 12th day: and all the cells in the body of a woman can be divided into 2 classes - those with an active maternal X chromosome and those with an active paternal, according to what happened to their ancestor cell at the time of Lyonisation.

Inactivity of the Barr-body-producing X chromosome is not indicated only by the condensed condition of the chromatin (preventing R.N.A. - messenger coding and thus participation in protein synthesis) in contrast to autosomes and the active isopyknotic X

(Gilbert et al., 1962). D.N.A. - labelling experiments showed that the Barr-body X, synthesises its D.N.A. at the end of the S-period, remaining "hot" after the autosomes and the active X have completed their synthesis (Gilbert et al., 1962; Rowley et al., 1963); and this is thought to be caused by the need for uncoiling the condensed chromatin before D.N.A. synthesis can begin.

At the time when Lyon published her views, studies were independently under way by Beutler and his colleagues considering the possiblity that the human female represented a mosaic of cells consisting of those with an active paternal X chromosome and those with an active maternal X chromosome. Whereas Lyon based her hypothesis on the mosaicism of phenotypic expression in female mice heterozygous for X linked recessive genes affecting coat colour, Beutler et al., (1962) tested the same hypothesis by studying the red cells of females heterozygous for glucose-6-phosphate dehydrogenase (g-6-pd) deficiency. If such females represented a genetic mosaic, one would expect two red cell populations: g-6-pd-deficient cells and normal cells. If, on the other hand, both X chromosomes were active in all of their red cell precursors, a single population of red cells with intermediate g-6-pd deficiency would be predicted. The glutathione stability of red cells of heterozygotes (GSH) was

studied in 2 different systems: in each system, the disappearance of GSH in the presence of acetylphenyl-hydrazine followed the course predicted for, and demonstrated to occur with, a mixed red cell population consisting of g-6-pd-deficient and normal cells. Also the rate of reduction of methaemoglobin in the presence of Nile blue, followed the course predicted for, and demonstrated to occur with, a mixed red cell population of deficient and normal cells. These findings support the hypothesis that only one of the X chromosomes is acitve in each red cell precursor of the human female. They explained the markedly variable penetrance of this defect in females on the grounds of early inactivation of one of the X chromosomes.

Further support followed when Davidson et al., (1963) studied the same problem using skin culture to produce clones derived from single cells from females heterozygous for the g-6-pd variants. From a quantitiative (assay) and a qualitative (electrophoretic study), they concluded that, with respect to the g-6-pd locus, there were indeed two distinct populations of cells in the heterozygous female. This was direct evidence in favour of the "Lyon hypothesis" - in each single cell of the female only one g-6-pd locus was operative. The hypothesis, moreover, offered a long awaited explanation to the phenomenon called "dosage compensation", a term coined by Muller (1932) to account

for the equality of phenotypic expression in males and females for most genes located on the X chromosome. Thus, the inactivation of one, or at least part of one, X chromosome, is most probably the mechanism of dosage compensation.

A piece of discordant evidence, however, has emerged when studying women heterozygous for the sex-linked blood group Xg^a. It was expected that they should have a mixture of Xg(a+) and Xg(a-) red cells. In fact, a study by a flourescent antibody technique, demonstrated conclusively that all their red cells are Xg(a+) (Reed et al., 1963). But this was not fatal to the hypothesis: a segment of the Lyonised X chromosome may remain active; the antigen may be diffusible; or, one race of cells may have a selective advantage over the other (Lancet, 2,769, 1963).

When considering the various patterns of sex-chromosome anomalies, the picture becomes even more confused. The total inactivation of all x chromosomes beyond the first, assumed by Lyon's hypothesis in its extreme form, is obviously difficult to reconcile with the general picture of these anomalies. A train of explanations and hypotheses is at present continually appearing in the literature, relating to incomplete activation of X chromosomes, the paternal origin of the Barr-body-forming X chromosome, the post-zygotic differentiation of the Barr body and various other

aspects (Bateman, 1962; Muldal, 1962; Lancet, 2, 769, 1963: Demars, 1963). Although the pathogenesis of the sex-chromosome anomalies is still far from being completely understood, it seems extremely likely that the curious behaviour of the X, uncovered by Lyon, will prove an essential part of the process. It has been recently pointed out that this heteropyknotic behaviour of the X chromosome is probably under autosomal control (Ohno, 1963).

B. THE SEX RATIO

Interest in the differential foetal wastage in the human is old. Many attempts to determine the sex of embryos of a younger gestational age were made. Unfortunately, the appearance of the external genitalia is not reliable for this purpose before the 5 cm. stage (Szontagh et al., 1961). Wilson (1926) pointed out discrepancy between naked eye sexing and histological sex of the gonads earlier than the 12th postmenstrual week, there being a tendency to err towards the male side on account of the relatively large size of the clitoris. Former investigators based their findings on gonadal histology, but in the early stages (up to 17 mms.) of human development no indication as to the future sex of the embryo can be obtained by a study of the gonads since these organs are not yet identifiable

as testes or ovaries (Hamilton et al., 1962). Curves were plotted for the relative incidence of each sex as pregnancy went on, and the sex ratio of earlier embryos was deduced by extrapolation of the curves (Pfaundler, 1936). The advent of nuclear sexing was therefore welcomed, and by examining the foetus or placental tissue, it was possible to diagnose the sex of 10 - 12 days embryos (Park, 1957). This was naturally followed by the appearance of various reports on the sex ratio in abortion material. Some of these were cited by Stevenson (1961) as shown in Table XV, and our own findings are included for comparison. Stevenson rejected the results of Bohle and Heinz on the grounds that they had used old sections collected for routine histology and not specially fixed or stained. Excluding their figures, Stevenson pointed out that the overall sex ratio for the various series was 142 males to 100 females, which is very close to our figure of 140.47 to 100. The distribution by gestation age in our sexed abortions was known in 189 specimens shown in Table XVI, together with the two series of Stevenson (1959) and Tricomi et al., (1960). As pointed out by Tricomi et al., (1960), no correlation was found between the sex ratio and the gestation age. It follows therefore, that the method used by former workers to calculate the primary sex ratio, that is the sex ratio at conception, by plotting curves for various months and

Authors	No. of concepti sexed		Sex ratio
	Male	Female	M/F
Bohle et al. (1957)	470	175	2.68
Stevenson (1959)	204	152	1.34
Esposito (1958)	11	9	1.22
Klinger et al (1958)	21	22	0.95
Heinz (1959)	556	169	3.30
Tricomi et al (1960)	149	93	1.60
Wagner (1958)	81	45	1.80
Glenister (1956)	16	19	0.84
Total	1508	684	2.20
Present series	118	84	1.40

TABLE XV. Results of nuclear sexing of products of conception in pre-28th week products of conception.
After Stevenson (1961) - my results included for comparison.

Month	Stevenson (1959)			Tricomi et al. (1960)			Our series		
	Males	Females	Sex ratio	Males	Females	Sex ratio	Males	Females	Sex ratio
1	15	18	183	3	7	43	0	0	-
2	95	70	136	22	13	169	8	7	114
3	63	42	150	72	41	176	23	22	104
4	20	17	118	39	24	162	22	17	129
5	7	5	140	10	7	143	28	11	254
6	3	0	-	0	0	-	24	11	218
7	0	0	-	0	0	-	7	9	78

TABLE XVI. Sex distribution by month of conception in our series as well as in two series of Stevenson (1959) and Tricomi et al. (1960).

extrapolating the results, was not correct.

The estimation of the primary sex ratio has engaged the interest of many workers for well over a century. Although their data are open to question, former investigators believed that the sex ratio at the time of conception (the primary sex ratio), differed decidedly from that at birth (the secondary sex ratio), in the direction of a much higher proportion of males. The secondary sex ratio is fairly stable, and is generally accepted to be 106 males to 100 females. Within the period of this investigation, 4000 consecutive deliveries at the S.M.M.P., R.I.E., consisted of 2089 boys and 1924 girls, giving a secondary sex ratio of 108.46/100, in Edinburgh. Whatever the primary sex ratio may be, it must somehow be brought at term to exactly the fairly constant secondary sex ratio.

At the present day, the question of the primary sex ratio seems to have more than academic interest, as it may throw some light on certain sex-linked lethal abnormalities or mutations in Man (Stevenson and McClarin, 1957). It is clear that the direct establishment of the primary sex ratio in Man is at present impracticable. This would require, among other things, the recovery and determination of sex of zygotes which fail to cleave and blastocysts which fail to implant. However, with the additional data that could be arrived at by resort

to nuclear sexing, an approximation of the primary sex ratio could be attempted which was heretofore not possible (Tricomi et al., 1960). Accepting the secondary sex ratio to be 106/100, and assuming that the general incidence of abortion is 10 per cent, Tricomi and his colleagues (1960) attempted to estimate the primary sex ratio by calculation, using a weighted mean. In their abortion series the sex ratio was 160/100. It follows therefore that in one-tenth of pregnancies the sex ratio is 160:100, while in nine-tenths the ratio is 106:100. From these data, they derive the following formula for the primary sex ratio: $\frac{(160x1) + (106x9)}{10}$ This gives a primary sex ratio of 111:100.

This calculation, admirable as it is, may represent the nearest figure to the primary sex ratio. One, however, remains confronted with two important questions, which make it difficult to accept the views of Tricomi and his colleagues:

1. Is the abortion incidence really 10 per cent of pregnancies?

2. What about the very wide range of the sex ratio in abortions as diagnosed by nuclear sexing, given by various workers. As shown in Table XV, even when we consider series of 200 or more only, a range of 140-330/100 is found.

Moreover, this application of nuclear sexing, can be criticized as inaccurate because it does not

take into account the various intrinsic and extrinsic factors underlying abortion.

Another approach to the question seemed to promise more accurate information, namely to study the sex of ectopic pregnancies. These represent ova which soon after ferilization have implanted themselves (in the tube) because of purely extrensic (tubal) factors that have nothing to do with the sex of the fertilized ovum. It is therefore reasonable to assume that male and female ova are present in ectopic pregnancies in a ratio that is very close to that at fertilization.

For the purpose of this study, I reviewed histological preparations from 120 cases of tubal pregnancy, and selected 45 cases that were found suitable for nuclear sexing. In these, the chorionic villi contained a sufficient number of nuclei in a sufficient state of preservation to show the sex chromatin if present. Fresh paraffin sections were specially cut and stained. In this series, 19 cases were diagnosed as female and 26 as male, giving a sex ratio of 126.3/100 (Hathout, 1963). This was later supplemented by 4 fresh cases, 2 male and 2 female, giving a sex ratio of 133.3/100. Both figures are very close to the ratio of 129/100 reported by Wagner (1958) in his series of 94 tubal pregnancies.

Ever since the question of the primary sex ratio was studied, no matter by what method, a definite

male preponderance has been a constant finding. If larger series of ectopic pregnancies continue to support this male preponderance, one would have to consider two possibilities. The first is that the male conceptus, being subject to more wastage, has to be produced in excess, this being Nature's attempt to secure equal numbers of men and women. The second possibility is that apparent male preponderance with later over-wastage is preceded with a much earlier loss of female ova so far unassessed (Ludwig and Boost, 1940). Even if such very early ova were procurable, not all of them could be nuclear sexed, for the sex chromatin, appears only after 12 - 19 days from fertilization (Barr, 1962). Stevenson, (1961) doubts very much that it is possible that such an excess of female losses can occur in the first 14 days of life as to balance all the subsequent excess of males estimated to be in the uterus at the end of that period.

Some authors argue that according to the laws of heredity the primary sex ratio must be 1:1 (Heuser, 1958). Logical as it is in theory, accumulating data show that this view does not operate in reality. Bishop (1960), maintains that the production of X and Y bearing spermatozoa is not always equal, that differential selection of X or Y bearing spermatozoa does take place in the female genital tract and that their ability to fertilize the ovum is not equal. Shettles (1961),

maintains that the less the time of insemination coincides with ovulation, the less favourable are the characteristics of the cervical mucus, and this is more readily penetrated by the large-headed X chromosome-containing spermatozoon, which is more vigorous and longer-lived than the Y chromosome-containing spermatozoon. On the other hand, inseminations that take place very close to the time of ovulation, when the cervical mucus is most favourable because of its fluidity, carry a much better chance for the lighter-weighted and more easily mobile Y chromozome-containing spermatozoon. Moore and Price (1948) produced evidence that the sex-ratio performances (S.R.P.) are affected by the pH of the male's blood serum, which in itself is subject to such influences as the stress of high altitude. Moreover, statistical data have always pointed out the existence of all-boys or all-girls families (Geissler, 1889), and seasonal variations of the sex ratio (Slatis, 1953).

It is interesting to reckon that the fragility of the male sex, manifested by antenatal male overwastage, continues to operate after birth. Since the stillbirth and neonatal mortality is higher in males, the sex ratio of 106:100 at birth is reduced in the first year to about 103:100 for all infants under 1 year of age. It is gradually decreased still further to approximately 100.7:100 for the general population at all ages in

U.S.A. (United Nations, 1949-50). "Nature" seems to have attained the desirable goal of providing men and women in equal numbers at adult age, but why this is done by gradually reducing a much higher sex ratio commencing at conception, is still one of Nature's mysteries.

PART 5

Chromosome Studies.

Observations

When the plan of this work was being drawn, it was hoped that chromosomal studies would proceed hand in hand with the other investigations. This, however, proved impracticable, as the equipping and establishment of the cytology laboratory was yet to be done. Even then, the development of reliable techniques proved to be time consuming. Experience had to be acquired and many minor details were learnt only by repeated trial and error.

Seven foetuses have been studied, a brief comment on each will be given. Table XVII shows the chromosomal findings.

Specimen 1.

The maternal age at abortion was 44 years, the mother had 4 living children and 2 abortions previously. The foetus was spontaneously aborted at 23 weeks of gestation age. It was a female foetus, not macerated, and externally showed a flexion deformity of the right wrist and bilateral talipes. Internally, there was a persistent mesentry of the descending colon. The umbilical cord showed 2 abnormalities: a single umbilical artery and a velamentous insertion. The placenta was unhealthy. Sex-chromatin was positive (placenta). Skin was cultured and the cells were interpreted as normal

with a chromosome constitution of 44 + XX.

Specimen 2.

This was a male nonmacerated foetus spontaneously aborted. The mother was a Para 2 aged 27 years. Gestation age was 20 weeks. Anatomical findings were normal, but the placenta was diagnosed as placenta praevia. Sex-chromatin negative. Skin cultures revealed a normal male karotype of 44 + XY.

Specimen 3.

The mother was a primigravida aged 35 years with history of spinal tuberculosis; she was not married. The foetus was a nonmacerated female, of 23 weeks gestation age, and showed no abnormalities. The placenta was partly extrachorial. Skin cultures showed a normal female karyotype of 44 + XX.

Specimen 4.

Maternal age at abortion was 40 years. Preceded by 2 abortions at 12 and 10 weeks but no viable pregnancies. Spontaneous abortion at 18 weeks. The foetus was a nonmacerated female with no anatomical malformations. The cord and placenta were normal. Sex-chromatin positive. Skin cultures showed a normal female karyotype of 44 + XX.

Specimen 5.

The mother was a Para 2 aged 28 years. Spontaneous abortion occurred at 14 weeks and the foetus was a nonmacerated normal female. The cord and placenta were normal. The sex-chromatin was positive. Cultures were set from fascia, and showed a normal female karyotype of 44 + XX.

Specimen 6.

The mother was a primigravida 24 years of age and married for 2 years. Spontaneous abortion occurred at 25 weeks. The foetus was nonmacerated male with the following congenital anomalies.

i. Club left foot.

ii. Bilobed right lung.

iii. There was a deep sulcus on the heart demarkating the two ventricles in an arcuate fashion;
the pulmonary artery seemed to join the aorta directly instead of being connected by a ductus arteriosus. In other words, the ductus arteriosus was as big as, and in direct continuity with, the main pulmonary artery, the right and left pulmonary arteries arising from either side of the main trunk.

The placenta and cord were normal. The sex-chromatin

was negative. Fascial cultures were prepared and showed a normal male karyotype of 44 + XY.

Specimen 7.

The mother was an unmarried, engaged, primigravida 18 years of age. Both herself and her consort were twins. First seen at 17 weeks, her uterus was the size of 28 weeks. Four weeks later, the uterus was two fingers below the xiphisternum, she had a weight gain of 16½ pounds and had gross oedema of both lower limbs. Twins and hydramnios were diagnosed radiologically. At 23 weeks spontaneous abortion occurred, and the volume of liquor passed was reported to be more than seven pints. When received the cords had been cut, the placenta was a single placenta of uni-ovular twins, and the membranes were torn. The twins were nonmacerated males weighing 530.7 gms. and 390.7 gms. One of the cords was attached near the centre of the placenta and the other had a velamentous insertion. The cords anastomosed freely, but the area of supply of the central cord was larger, in the proportion of 1½:1. On autopsy, the larger foetus was shown to have a Mackel's diverticulum and dilated tortuous ureters contrasting to those of its normal twin. Both twins were sex-chromatin negative. Skin cultures were set but only the preparations from the larger twin were satisfactory. They showed a normal male karyotype of 44 + XY.

Specimen no.	Chromosome-count distribution						Total	Karyotype
	44	45	46	47	48	Polyploids		
1	1	1	22	1	0	0	25	44 + XX
2	0	2	19	1	0	1	23	44 + XY
3	1	0	20	1	0	1	24	44 + XX
4	0	1	19	1	0	0	21	44 + XX
5	0	0	26	0	0	0	26	44 + XX
6	0	0	30	0	0	0	30	44 + XY
7	1	1	20	1	0	0	21	44 + XY

TABLE XVII. Summary of the findings.

Discussion

Historical

The study of mammaliam chromosomes lagged behind the same studies in insects and plants until fairly recently. Students of avian and mammalian chromosomes had always been handicapped by the fact that most species possess a large number of small chromosomes which usually crowd the metaphase plate and make counting and observation of individual chromosomes very difficult. Early workers used material obtained from cadevers where post-mortem changes gave rise to clumping of the chromosomes (Evans and Swezy, 1929). Painter (1923) recommended the use of freshly biopsied material which was immediately fixed. The finest material was that obtained from executed criminals, removed at the foot of the gallows and fixed in less than 1 minute (Chu, 1960). In spite of tremendous technical difficulties, those early investigators obtained remarkable results.

Ever since it became known to biologists that chromosomes were constant in number for a given species of animal or plant (cf. Stern, 1959), curiosity was aroused as to the chromosome number of Man. The first attempt to count them was that of Hansemann (1891) who reported cells with 18,24 and more than 40 chromosomes. Other reports followed, with a range from 16 to 32. In 1912, De Winiwarter reported 47 chromosomes at meta-

phase in spermatogonia and 23 autosomal bivalents plus an unpaired X in primary speratocytes. In 1917, Wieman made the first report of the presence of XY chromosomes. In 1918, Evans was the first to find a diploid number of 48 chromosomes in spermatogonia. In 1921, Painter reported the presence of a small Y chromosome in males; he stated that "the counts ranged from 45 to 48 apparent chromosomes, although in the clearest equatorial plates only 46 chromosomes were found," and concluded that the diploid chromosome number in Man was either 46 or 48. Painter, later (1923), came to the conclusion that the correct diploid number was 48; but this conclusion was based on the study of testicular biopsies from 3 mental patients, and it remains unknown whether they had chromosomal variations. Painter also reported on the occurrence of tetraploid cells, and suggested the possibility of an XXY hermaphrodite originating in a similar manner to the findings of Bridges (1922), in which XXY flies were derived from triploid Drosophila. For a long time after, it was taught that the diploid number of chromosomes in Man was 48, and the value of 2n = 48 was generally accepted.

Technical Advances

It was the rapid development of tissue culture

techniques that made the study of human chromosomes both easy and accurate. It became possible to observe and photograph human cells in the living state. The fact that the growth zone consists only of a single layer of cells (monolayer, Fig. 108) facilitates experimental treatments as well as cytological fixation and staining. Mitotic activity is usually more enhanced in vitro than in vivo, and it can be subjected to experimental control. As Chu (1960) also points out, cell cultures have a great advantage over sectioned histological preparations, which were used exclusively in earlier studies of human chromosomes, in that cells in culture are more flattened and stretched on the substrate and no cellular material is lost or added by sectioning. Furthermore, it became possible to study the chromosomal complement of various tissues of the same person, and thus reveal mosaicism if present. Although tissue culture is subject to criticism on account of the known phenomenon of karyotypic changes which occur in vitro (Hsu, 1959), the question does not arise in short-term cultures such as those of bone marrow cells, and techniques are now available by which it is possible to maintain a euploid condition of human cell lines for a considerable time without obvious chromosomal alterations.

The object of all techniques for chromosome study is to obtain numerous intact, well fixed, dividing

cells which are large, easily flattened and contain separate, analysable chromosomes (Clarke, 1962). The various aspects of tissue culture and details of collecting and storage of tissue before culture, precautions to avoid contamination, etc. have been reviewed by Paul (1960). The main principles are to allow the cells to grow as a monolayer in a special environment, put them in suspension by trypsinization, and expose them to colchicine which acts as a spindle inhibitor (Eigsti and Dustin, 1955) and also increases the number of metaphases (Tijio and Levan, 1956; Ford and Hamerton, 1956a), shortening chromosomes which otherwise overlap and spreading the chromatids for ease in determining the position of the centromere. To achieve chromosome dispersion, the cells are treated by a hypotonic solution (Slifer, 1934; Makino and Nishimura, 1952). The cells are then fixed, and spreading of the chromosomes in one plane is achieved by drying over a flame (Tjio and Puck, 1958a). After staining and mounting the cells are ready for cytological examination.

The Normal Human Karyotype

Early in 1956, Tjio and Levan, working in Sweden, made the surprising announcement that they consistently found counts of 46 chromosomes in human tissues. Their preparations were made from tissue cultures set up from lung explants obtained from aborted

foetuses. Their preparations were of such a high quality as to testify the correctness of their conclusions, and they revealed the chromosomes of the diploid somatic complement with a degree of detail hitherto unknown. Soon after, the diploid number of 46 was confirmed by the finding of Ford and Hamerton (1956b) of 23 bivalents in diakinesis and metaphase of primary spermatocytes in testicular material. Since then, various concordant reports followed (Ford et al. 1958; Tjio and Puck, 1958b; Chu and Giles, 1959; Moorhead et al. 1960). These and many other studies, using the modern methods for the study of chromosomes, leave no remaining doubt that there are 46 chromosomes in the normal somatic cells of normal men and women (Ford, 1962).

The only reports that contradicted with the accepted figure of 46 chromosomes, were those of Kodani (1957, 1958) and Chang (1960). Kodani, though accepting the basic figure of 46, belived that there were sometimes supernumerary chromosomes in man, resulting in chromosome counts of 46, 47 and 48. Chang also claimed to have found 48 chromosomes in several Chinese; in his studies he used the old method of sectioning. The views of Kodani and Chang were critically discussed (Chu, 1959; Ford, 1962), and the consensus at the present time accepts 46 as the normal human chromosomal complement.

In 1959 Chu and Giles reported that every pair of homologous chromosomes of the human complement could

be individually recognized. Furthermore, statistical analysis indicated that homologous autosomes from cells for the same or different individuals did not differ significantly either in their relative length or in the position of the centromere. The only difference between chromosome complements of males and females was in the sex chromosomes. There were no significant differences among the X chromosomes or among the Y chromosomes from different individuals (Chu, 1959).

Attempts to define the individual members of the human diploid somatic set have been made, based mainly on two primary features: the relative length and the centromere position. Other factors were considered such as the presence of satellites on the short arms of the chromosomes, the presence of constrictions on the long arms and the angle of divergence of the chromatids, but experience showed that these were variable and could not be relied on (Ford, 1962). Up to the present moment, it has not been possible to identify each one of the 46 chromosomes individually, but they could be broken up into identifiable groups of chromosomes. Various classifications were done which were all in agreement but differed only in nomenclature. In 1960 a study group met at Denver and recommended the universal use of the classification known as the Denver System (Lancet, 1960); which they summarised as follows, designating each group by the arabic numerals of the extreme chromo-

somes of the group joined by a hyphen:-

Group 1 - 3 Large chromosomes with approximately median centromeres. The three chromosomes are readily distinguished from each other by size and centromere position.

Group 4 - 5 Large chromosomes with submedian centromeres. The two chromosomes are difficult to distinguish, but chromosome 4 is slightly longer.

Group 6 - 12 Medium sized chromosomes with submedian centromeres. The X chromosome resembles the longer chromosomes in this group, especially chromosome 6, from which it is difficult to distinguish. This large group is the one which presents major difficulty in identification of individual chromosomes.

Group 13 - 15 Medium-sized chromosomes with nearly terminal centromeres ("acrocentric" chromosomes). Chromosome 13 has prominent satellites on the short arms. Chromosome 14 has small satellites on the short arms. No satellites have been detected on chromosome 15.

Group 16 - 18 Rather short chromosomes with approximately median (in chromosome

16) or submedian centromeres.

Group 19 - 20 Short chromosomes with approximately median centromeres.

Group 21 - 22 Very short, acrocentric chromosomes. Chromosome 21 has satellites on its short arms. The Y chromosome is similar to these chromosomes.

As already pointed out (Ford, 1962), further observations did not support the reliance upon satellites as a means of discrimination, and it was abandoned by most workers.

This system has the advantage of flexibility, but its use seems to be cumbersome in certain circumstances - for instance the expression of a reciprocal translocation between undefined members of two separate groups. For this purpose, a system of lettering like that employed by Lejeune and his group (1959) would be more convenient. Unfortunately the French system, which has priority, does not lend itself to be readily used in conjunction with Denver numbering. The lettering system of Patau et al. (1961) is compatible with the Denver system: indicating the seven groups by the letters A to G. Thus it is easier to refer to a D - G translocation, instead of saying that there is translocation between a chromosome from Group 21 - 22 and another from Group 13 - 15. Hauschka (Ford, 1961) advocated the

combination of Denver numbering with Patau letters: so that Group 1 - 3 would be called A1, A2 and A3, and so on whenever individual chromosomes could be unequivocally distinguishable.

Fig. 109a and b shows a normal male karyogram; Fig. 110 a and b shows that of a normal female.

Chromosomal Abnormalities

A. The Sex Chromosomes

As soon as the techniques for studying human chromosomes were established, various publications started to appear in the literature, referring to a number of abnormal chromosomal constitutions associated with well known clinical syndromes. The majority of these relate to the sex chromosomes. Jacobs and Strong (1959) produced evidence of an XXY sex chromosome complement in an apparent male who showed the features of Klinefelter's syndrome. Ford et al. (1959) made the first description of an XO sex chromosome complement in a patient with gonadal dysgenesis (Turner's syndrome). Jacobs and her colleagues (1959a) were the first to describe a woman with an XXX sex chromosome complement (the so-called superfemale), and also (1959b) showed that women with the features of testicular feminization were chromosomal males with XY sex chromosomes. Ford

(1959) reported the XO/XX mosaics. In 1960, Jacobs and her colleagues reported the first instance of a female patient who had a morphological (versus numerical) abnormality of an X chromosome, in the form of deletion of the long arm of one X chromosome; they are designated X^X females, have a low sex-chromatin count with a proportion of the bodies being smaller than normal. In the same paper, Jacobs and her colleagues described the first example of XO/XXX mosaicism. Fraccaro et al. (1960) described women with one normal X chromosome, the other being an isochromosome for the long arm of an X (XX); And Blank et al. (1961) described the same complement in mosaicism with XO (XO/XX). In 1961, Hayward and Cameron reported a case of XO/XX/XXX mosaicism, the patient being a female child with some features of Turner's syndrome and Hirschsprung's disease. Jacobs et al. (1961a) described XΔ females, with a deletion of the short arm of one X chromosome. They also reported XO females who were sex-chromatin positive; their smears containing an unusually small Barr body, the possibility being either a reduplication of material on one or other arm of the single X, or the translocation of some X chromosome material to an autosome, the change in either instance being too small to be observable. In the same report, Jacobs et al. described the XO/XY and XO/XYY female mosaics. Bahner's report of a fertile chromatin-positive

XO female with features of Turner's syndrome was referred to in Part 4 of this work.

The significance of supplementing chromosomal analyses with sex-chromatin studies in the diagnosis of X chromosome anomalies has already been referred to. It is important to remember than in mosaicism due to post-zygotic nondisjunction, the buccal smear may not be representative of the situation in other tissues, as is the case in XO/XX, XO/XXX and XO/XX females where XO cells constitute the buccal mucosa (Court Brown and MacGregor, 1963).

In apparent males with Klinefelter's syndrome, the original report of Jacobs and Strong (1959) of XXY complement, was followed by various others, which revealed the same anomaly as well as other chromosomal variants in Klinefelter's syndrome, such as XX/XXY mosaicism (Ford et al., 1959b), XXYY (Muldal and Ockey, 1960; Carr et al., 1961), XXXY (Barr et al., 1959), and XXXXY (Fraccaro and Lindsten, 1960). Cases of chromatin negative Klinefelters syndrome with a normal XY complement were also described (Court Brown et al., 1960). The association between Klinefelter's syndrome and mongolism was reviewed by Polani (1962.)

Much light has been thrown on the function of the Y chromosome. It is known to be positively male-determining, and it seems to be capable of showing the effect of its presence in the face of two, three and

even four X chromosomes: although of course testicular tissue formed under these conditions is far from being normal. There are, however, exceptions to the testis determining role of the Y chromosome, for instance the intersexual male with testicular tissue and an XO sex chromosome constitution, and the true hermaphrodites in whom testicular tissue exists in presence of an XX sex chromosome complement.

It must be remembered that the sex chromosome complement is but one aspect of the complex mechanism of sex development. The autosomal drive, the organizing role of the gonads, and other environmental factors, such as the hormone balance, have got a definite influence; and certain abnormalities of sex development are due to abnormalities in these factors (Jeffcoate, 1963).

B. Autosomal Abnormalities

The first autosomal abnormality to be discovered in Man, is the trisomy associated with mongolism (Lejeune et al., 1959b). The commonest anomaly (regular mongol), is trisomy 21 - 22, usually considered to be 21. In about 5 per cent of cases, the extra chromosome is translocated to another chromosome in the 13 - 15 group (translocation mongol) (Carr, 1962; Penrose et al. 1960), in which the chromosome count is apparently 46, carrying the chromosomal material of 47 chromosomes. In a few instances their is mosaicism; some cells have a normal chromosome complement, others having an extra chromosome 21, which may account for the incompleteness of the clinical manifestations of the syndrome, that is often encountered (Moore and Hay, 1963). Another explanation to this, is that part of the extra chromosome 21 is deleted (Ilbery et al., 1961). Some mosaics may have normal findings in leucocytic cultures so that further studies e.g. of skin cultures are necessary for diagnosis (Clarke et al., 1961).

The well known association of increased maternal age with mongolism in the offspring, suggests that the underlying factor is nondisjunction during oogenesis (Carr, 1962; Maclean et al., 1962; Penrose et al., 1960). It used to be difficult to understand why young mothers occasionally produced mongols, but this problem has been

solved to a great extent by the knowledge that a young parent (father or mother) may be a carrier of a translocation chromosome (Carr, 1962). The chromosome count of such a translocation carrier is apparently 45, but these represent the chromosome material of a normal set of 46 chromosomes: therefore these carriers do not show evidence of the disease, but are capable of passing the abnormal chromosome to their children. The chances are that the offspring may be normal, carrier, or mongol (familial mongol), in the proportion 2:1:1, if one parent is a carrier and the other is normal. Chromosome analysis of the parents and siblings should therefore be part of the investigation of mongols, especially those born to young mothers. Such analyses give vital information necessary for sound genetic counselling (Moore and Hay, 1963).

Another well defined, clinically distinguishable, trisomic syndrome is <u>trisomy 17 - 18</u>. The clinical features are: mental deficiency, small deformed mandible, low set malformed ears, webbed neck, flexion deformity of the fingers, short big toe, deficient falx cerebri and congenital heart disease; other minor abnormalities may also be found. Edwards et al. (1960) believe the extra chromosome to be number 17, whereas Smith et al. (1960) refer to similar cases as examples of trisomy 18. It is doubtful if the separation is real, for it is

difficult to distinguish between chromosomes 17 and 18 (Lancet, 1961).

Trisomy of one of the chromosomes of the 13 - 15 group was first described by Patau et al. (1960) in a female infant with multiple congenital malformations. They reported other cases with the same chromosomal abnormality, and their views were confirmed by other workers (Ellis and Marwood, 1960). The features of this syndrome are: mental deficiency, eye defect, hyperconvex finger nails, cleft palate, cleft lip, haemangiomata, horizontal palmer creases and interventricular septal defect. In this syndrome it is difficult to know which is the trisomic chromosome, but, on account of the concordance of the findings, it probably always affects the same chromosome (Moore et al., 1963). More recently, Zellweger et al., (1962) described 2 cases of multiple congenital anomalies with partial trisomy 13 - 15.

Other incidents of autosomal trisomy have been described in association with various developmental anomalies, but an aetiological relation cannot be always established, since the same phenotypic anomaly was not invariably associated with the chromosomal abnormality. An example of these is trisomy 22 associated with a case of Sturge-Weber syndrome (Hayward and Bower, 1960).

Böök et al. (1961a) described a karyotype with probable trisomy of number 19 - 20 and monosomy of 22. Böök and Santesson (1960) described a triploid mosaic infant with developmental malformations (lipomatosis of the back of the hands, feet and thighs, micrognathia and syndactyly of the hands and feet), in whom there were two categories of cells: normal diploid cells and cells with a complete triploid set with an XXY combination.

Investigations have revealed the fact that in contrast to the sex chromosomes, numerical variations of the autosomes are rare. Court Brown (1962), pointed out that autosomal numerical aberrations almost always involve the addition of a chromosome, for the missing of a whole autosome cannot be tolerated. It is known that all the autosomes carry a number of recessive lethal genes, which, when balanced by normal alleles on the corresponding members of the homologous pairs, do not produce a lethal effect. If the normal alleles are lost, as they will be in a monosomic state, then the recessive lethals will no longer be recessive and will lead to the death of the organism. In a similar way, trisomic states may lead to such genetic imbalance in a cell as to be lethal. Both abnormalities are, therefore, more to be expected in abortion material than in live births.

Apart from numerical variations so far considered, structural abnormalities were discovered, such

as reciprocal translocations, pericentric inversions, deletions, reduplications, isochromosome formation, etc. If the chromosomal re-arrangements are such that the total genetic material is the same, they are known as balanced re-arrangements, and no obvious change in the phenotype of the individual is expected. But if genetic material is decreased or, indeed, increased in amount, then the change is unbalanced and phenotypic abnormalities are produced (Court Brown, 1962). The example of the parent of a mongol with a translocation chromosome has already been described. The first instance of translocation in man was, however, discovered by Turpin et al. in 1959; this was a translocation of chromosome number 22 to one of the 13 - 15 group, in a male infant with multiple malformations of the vertebrae. Lejeune et al. (1960) described a typical case of Klinefelter's syndrome with a chromosome count of 46 instead of 47. An analysis of the karyotype revealed the presence of only four long acrocentric chromosomes, and a new chromosome which was interpreted as the product of reciprocal translocation between two chromosomes of the 13 - 15 group. Böök et al. (1961b) described a translocation of a segment of the long arms of chromosome 2 on to the long arm of a chromosome of the 6 - 12 group. This was a balanced change in a normally developed girl, although her mother had an atrial septal defect and was a mosaic of 46 and 47 cells. Edwards and Clarke (1960) described translocation of the

greatest part of the short arms of chromosome 2 on to the long arm of chromosome 7, in a female patient with mental deficiency, unusual facies and ears, and hypotonia.

Incidents of chromosomal deletion and other structural abnormalities were cited by Fraccaro (1962). Edwards and Clarks (1960) described possible isochromosome formation for the long arm of chromosome number 6. Bain and Gauld (1963) reported a case of multiple congenital abnormalities with a ring chromosome formation.

The vast amount of literature reporting chromosomal studies of various human diseases and abnormalities is too wide and too fast to be quoted in full; but it leaves little doubt that cytogenetics used as a diagnostic tool, is opening new frontiers to our knowledge, and is doing that at a tremendous pace. One of the most notable advances in cancer research, was the discovery of the association between chronic myeloid leukaemia and specific chromosome abnormality, namely a deletion of material from the long arm of autosome 21. The initial discovery was made in Philadelphia by Nowell and Hungerford (1960), and much of the developmental work that followed has taken place in Edinburgh (Baikie et al., 1960; Tough et al., 1961), and it was the Edinburgh group who coined the name 'Philadelphia chromosome'. There seems little doubt that this change

arises 'de novo', and is not developmental in origin. The significance and implications of this discovery were discussed by Court Brown (1962).

The effects of radiation on human chromosomes following 'in vivo' exposure, were first reported by Tough et al. of Edinburgh (1960). They described a variety of structural abnormalities including chromosome fragments, ring chromosomes and dicentrics. The first reports followed the study of leukaemic patients treated by irradiation of the spleen. Retrospective studies are also being conducted in Edinburgh of patients who previously had radiation treatment for ankylosing spondylitis. It was shown that chromosomal structural damage can persist long after the treatment (Bender and Gooch, 1963). Patients receiving radiation for malignant tumours are also being studied. There is evidence to show that some of these changes are stable and capable of being perpetuated through mitotic division. It has been shown that therapeutic irradiation increases, at any rate probably for a period of 10 - 12 years, an individual's risk of developing leukaemia (or another form of cancer) in any one of these years. Great care should therefore be exercised in the exposure of patients in the treatment of nonmalignant conditions, and particularly the exposure of children (Court Brown, 1962).

It has been known that human cells allowed to multiply in vitro may undergo mitotic accidents, giving a variable rate of 'spontaneous' chromosomal aberrations (Fraccaro, 1960). The possibility of similar age changes occurring in vivo is to be suggested from the increased incidence of nondisjunction with increasing maternal age. Normal individuals are also liable to chromosomal changes, and Jacobs et al. (1963) found an increase in the number of aneuploid cells with age, in blood cultures from apparently normal individuals, and noticed a sex difference. These studies were made on a nonrandomly selected group of individuals. In a more recent study (1964), they made chromosome studies of a number of elderly people over 65 years of age, who were randomly chosen from the general population. Such a study would give more accurate information, and will provide a background against which to judge the various reports tending to associate apparently minor chromosome abnormalities with pathological states, for instance the abnormal chromosome described by Gunz (1962) in chronic lymphocytic leukaemia, or the familial chromosome abnormality associated with repeated abortions, described by Schmid (1962). Some of these associations, however, may be fortuitous, and an important aid to determining whether this is so will be the study of the frequency of minor abnormalities and variations in the general population. As a result of their study,

Jacobs et al. (1964) confirmed that in normal individuals in the general population, the incidence of chromosome abnormality increased with age. Harnden (1962) speculated on the accumulation of genetic change as a fundamental feature of the process of aging, and it seems that Jacobs et al. (1964) have provided some means for objectively measuring the process of aging. As Court Brown (1962) pointed out, these findings may bear a relation to the problem of carcinogenesis: it might well be the case that some of the harmful genetic changes that accumulate with age, either result in the initiation of a neoplastic process, or alternatively these may lead to provision of cells sensitive to the action of a carcinogen. The carcinogen itself may initiate neoplasia, or it may promote the emergence of new cell lines, some of which may ultimately acquire the actively invasive and autonomous features of clinical cancer.

Chromosomal Studies in Abortion Material

As already pointed out, a higher incidence of more major chromosomal abnormalities is to be expected in abortion material, abortion being the natural result of such abnormalities that are incompatible with the continuation of life. Relatively few studies, however, have been done in this field. Technical difficulties might be partly responsible for this (Naujoks, 1962), as well as the natural tendency amongst investigators to give priority to studying the human being in the living, or at least a more mature state.

The first reports of chromosomal anomaly in abortuses (Delhanty et al., 1961, Penrose and Delhanty, 1961) described triploid cell cultures in two cases. In the first, cultures had been set from the yolk sac and the limbs of a 9 week embryo, producing triploid cells with 69 chromosomes, and a few hexaploid cells. In the second, cultures from the gut and the yolk sac of a macerated foetus after missed abortion, producing two categories of cells, with 46 and 69 chromosomes. Penrose and Delhanty discussed triploidy in animals, both spontaneous and by means of artificial agents; they also pondered about the possibility of natural polyploidy of the amniotic cells. Klinger and Schwarzacher (1962), reported XY/XXY mosaicism in a 60 mm. sex-chromatin positive human foetus. Aula and Hjelt (1962) reported

a structural chromosome anomaly in 20 per cent of the cells cultured from a 120 mm. foetus.

Apart from these sporadic findings, the first series of abortions to be studied and reported was that of Makino et al. (1962). In cell cultures from 135 foetuses from therapeutic abortions, they found two chromosome anomalies: one had a translocation between two members of the 13 - 15 group, and the other had a high proportion of cells with chromosome breaks. Delhanty et al. (1963), studied 34 specimens from spontaneous abortions, and found two foetuses with 13 - 15 trisomy.

Carr (1963) reported his findings in a series of 35 spontaneous abortions and 6 stillbirths. The 35 spontaneous abortions were unselected; cultures from more abortions were attempted but failed (overall culture failure 30 per cent). The stillbirths, however, were highly selctive, because a request for tissue culture was usually received only if there were obvious anomalies. Carr's results were highly interesting. In none of the malformed stillbirths was there a chromosomal abnormality; but in 8 of the 35 abortions (almost 23 per cent) he reported definite chromosomal changes. He found 13 - 15 trisomy in three foetuses, XO sex chromosome complement in two foetuses, triploidy in one, 15 - 18 trisomy in one.

An additional series of 19 specimens studied by Carr and Willis (Carr, 1963), revealed one case of XO

sex chromosome anomaly, one case of 16 - 18 trisomy, one case of triploidy (with XXY sex chromosome complement) and one case of tetraploidy.

The findings in Carr's series certainly suggest that chromosomal abnormalities are a potent cause of early embryonic wastage. It is significant to note that he found the chromosome anomalies in early specimens; in each instance the embryo, if present, lagged in development behind that expected from the menstrual date of pregnancy, and the largest embryo found was about 25 mms., which puts it within the 2 months period. This is probably the stage at which to look for chromosomal abnormalities, and a search at a later period, as our small series suggests, will probably have missed them. The foetal age in our series ranged between 14 and 25 weeks. It is unfortunate that the two female foetuses reported in Part 4 of this work, the sex-chromatin negative one and the one with two sex chromatin bodies in a proportion of her cells, were not documented by chromosomal analyses. They were 13 and 16 weeks of gestation age, which is concordant with the fact that sex chromosome anomalies are not as lethal as autosomal anomalies, and it is doubtful whether the aetiology of the abortion in these 2 cases was chromosomal.

Carr (1963) is of the opinion that amniotic cultures reflect the chromosomal pattern of the embryo.

If so, then the study of blighted ova would certainly help in detecting chromosomal abnormalities that affect foetal development at a very early stage. It would be interesting to find out the incidence of chromosomal anomalies in spontaneous abortions of a very early stage and work in this direction is going on.

REFERENCES

AITKIN, N.B. (1958): Symposium on Nuclear Sex. Lond., 168.

ALLEN, F.J. (1892): J. Anat., 26, 300.

ALZAMORA, V. (1953): Pediatrics, 12, 259.

AREY, L.B. (1954): Developmental Anatomy. 6th ed., Saunders, Philadelphia & London, 105.

AULA, P., and HJELT, L. (1962): Ann. Paediat. Fenn., 8, 297.

BACSHICH, P., and RIDDEL, W.J.B. (1945): Nature, 155, 271.

BAHNER, F., SCHWARZ, G., HIENZ, H.A., and WALTER, K. (1960): Acta Endocr. (Kbh), 35, 397.

BAIKIE, A.G., COURT BROWN, W.M., BUCKTON, K.E., HARNDEN, D.G., JACOBS, P.A., and TOUGH, I.M. (1960): Nature, 188, 1165.

BAIN, A.D., and GAULD, I.K. (1963): Lancet, 2, 304.

BALLANTYNE, J.W. (1904): Antenatal Pathology and Hygiene. The Embryo. William Green & Sons, Edinburgh.

BARR, M.L., and BERTRAM, E.G. (1949): Nature, 163, 676.

BARR, M.L., and CARR, D.H. (1962): Chromosomes in Medicine. Ed. J. Hamerton. Little Club Clinic. Dev. Med. 5, National Spastic Society & Heinemann, Lond.

BARR, M.L., SHAVER, E.L., CARR, D.H., and PLUNKETT, E.R. (1959): J. Ment. Defic. Res., 3, 78.

BARTHOLOMEW, R.A., et. al. (1951): Amer. J. Obstet. Gynec., 62, 246.

BATEMAN, A.J. (1962): Lancet, 2, 1383.

BECKETT, R.S., and FLYNN, F.J. (1953): New England J. Med., 249, 345.

BENDER, M.A., and GOOCH, P.C. (1963): Radiation Research, 18, 389.

BENIRSCHKE, K., and BOURNE, G.L. (1960): Amer. J. Obstet. Gynec., 79, 251.

BENIRSCHKE, K., and BROWN, W.H. (1955): Obstet. & Gynec., 6, 399.

BERENGARIUS, J. (1521): Commentaria cum Amplissimis Additionibus super Anatomiam Mundini. Bologna.

BERGE, Ten, B.S. (1962): Geburtsh. Frauenheilk., 22, 1228.

BERGMAN, P., LUNDIN, P., and MALMSTRÖM, T. (1961): Acta Obstet. Gynec. Scand., 40, 372.

BEUTLER, E., YEH, M., and FAIRBANKS, V.F. (1962): Proc. Nat. Acad. Sci., 48, 9.

BISHOP, M.W.H. (1960): Memoirs of the Society for Endocrinology, No. 7 Ed. C.R. Austin. Cambridge University Press, 89.

BOHLE, A., STOLL, P., and VOSGERAU, H. (1957): Klin. Wschr., 35, 358.

BOOK, J.A., and SANTESSON, B. (1960): Lancet, 1, 858.

BOOK, J.A., SANTESSON, B., and ZETTERQUIST, P. (1961a): Acta Paediat. (Uppsala), 50, 217.

BOOK, J.A., SANTESSON, B., and ZETTERQUIST, P. (1961b): Lancet, 1, 167.

BOTELLA LLUSIA, J., and MARIN BONCHERA, E. (1961): Sem. Med. (B. Air.), 119, 1584.

BOURNE, G.L., and BENIRSCHKE, K. (1960): Arch. Dis. Childh., 35, 534.

BOYD, J.D. (1959): Ulster Med. J., 28, 35.

BOYD, J.D., and HAMILTON, W.J. (1960): J. Obstet. Gynaec. Brit. Emp., 67, 208.

BOYD, J.D., and HAMILTON, W.J. (1964): J. Obstet. Gynaec. Brit. Cwlth, 71, 1.

BRIDGES, C.B. (1922): Amer. Natur., 56, 51.

BROWNE, D. (1933): Practitioner, 131, 20.

BROWNE, F.J. (1925): J. Obstet. Gynaec. Brit. Emp., 32, 17.

BURDACH, D. (1758): De Laesione Partium Foetus Nutritione Inservientium. Lipsiae.

BURSZTEIN, R.P. (1962): Bull. Soc. Roy. Belg. Gynec. Obstet., 32, 23.

BUSCHAN, G. (1934): Deutch. Med. Wchnschr., 60, 410.

CAMPBELL, W.A.B. (1953): Lancet, 1, 173.

CARPENTIER, P.J., STOLLE, L.A.M., and VISSCHERS, G.P. (1955): Lancet, ii, 874.

CARR, D.H. (1962): Canad. Med. Ass. J., 87, 490.

CARR, D.H. (1963): Lancet, 2, 603.

CARR, D.H., BARR, M.L., and PLUNKETT, E.R. (1961): Canad. Med. Ass. J., 84, 873.

CHU, E.H.Y. (1950): Am. J. Hum. Genet., 12, (1), 97.

CHU, E.H.Y., and GILES, N.H. (1959): Amer. J. Hum. Genet., 11, 63.

CIBA FOUNDATION (1960): Symposium on Congenital Malformations. Ed. G. Wolstenholme, and C.O'Connor. Churchill, London.

CLARKE, C.M. (1962): Chromosomes in Medicine. Ed. J. Hamerton. Little Club Clinic Dev. Med. 5, National Spastics Society and Heinemann, London.

CLARKE, C.M., EDWARDS, J.H., and SMALLPLICE, V. (1961): Lancet, 1, 1028.

COFFEY, V.P., and JESSOP, W.J.E. (1959): Lancet, 2, 935.

CORNER, G.W. (1923): Am. J. Anat., 31, 523.

CORNER, G.W. (1944): Curselves Unborn. New Haven.

CORNER, G.W., and BARTELMEZ, G.W. (1953): Pregnancy Wastage. Engle, E.T., C.C. Thomas, Springfield, Ill., 6.

COURIE, V., and SLATER, E. (1963): Acta Genet. (Basel), 13, 77.

COURT BROWN, W.M. (1962): Symposium: Genetics in Medicine. Royal College of Physicians, Edinburgh.

COURT BROWN, W.M., and MacGREGOR, T.N. (1963): Modern Trends in Gynaecology. Ed. R.J. Kellar, Butterworths, London.

COURT BROWN, W.M., JACOBS, P.A., and DOLL, R. (1960): Lancet, 1, 160.

CREW, F.A.E. (1962): Genetical aspects of child health, in Child Health and Development. Ed. R.W.B. Ellis. 3rd ed., Churchill, London.

CULLING, C.F.A. (1957): Handbook of Histopathological Technique. Butterworth, London, 194.

CURTIS, A.H. (1925): J.A.M.A., 84, 1262.

DAVIDSON, R.G., NITOWSKY, H.M., and CHILDS, B. (1963): Proc. Nat. Acad. Sci., 50, 481.

DAVIDSON, W.M., FOWLER, J.F., and SMITH, D.R. (1958): Brit. J. Haem., 4, 231.

DAVIDSON, W.M., and SMITH, D.R. (1954): Brit. Med. J., 2, 6.

DAVIES, T.S. (1963): Brit. Med. J., 5344, 1541.

DEKABAN, A.S., CONE, T.E., RIVA, H.L., and LIEBERMAN, J.E. (1962): Am. J. Obst. & Gynec., 83, 532.

DELHANTY, J.D.A., ELLIS, J.R., and ROWLEY, P.T. (1961): Lancet, 1, 1286.

DE LEE (1913): Obstetrics. 1st ed. Saunders, Phil.

DE MARS, R. (1963): Science, 141, 649.

DEMPSEY, E.W., and WISLOCKI, G.B. (1944): Endocrinology, 35, 409.

DEVITT, R.E., and KENNY, S. (1962): Lancet, 1, 430.

DE WINIWARTER, H. (1912): Arch. Biol., 27, 91.

EASTMAN, N.J., and HELLMAN, L.M. (1961): Williams Obstetrics. 12th ed., Appleton Century Crofts, Inc., New York.

ECKMAN, T. R., and CARROW, L.A. (1962): Amer. J. Obstet. Gynec., 84, 222.

EDEN, T.W. (1897): J. Path. Bact., 4, 265.

EDMONDS, H.W. (1954): Amer. J. Obstet. Gynec., 67, 102.

EDWARDS, J.H. (1958): Brit. J. Prev. & Social Med., 12, 115.

EDWARDS, J.H., and CLARKE, G. (1960): Human Chromosome Newsletter, No. 2.

EDWARDS, J.H., HARNDEN, D.G., CAMERON, A.H., CROSSE, V.M., and WOLFF, O.H. (1960): Lancet, 1, 787.

EIGSTI, O.J., and DUSTIN, P. (1955): Colchicine in Agriculture, Medicine, Biology and Chemistry. Ames: Iowa, Iowa State College Press.

ELLIS, J.R., and MARWOOD, J.C. (1960): Human Chromosome Newsletter, No. 2.

ELLIS, R.W.B. (1963): Diseases In Infancy and Childhood. Livingstone, Edinburgh, 113.

EVANS, H.M. (1918): Genetics in Relation to Agriculture. McGrawhill, 538, footnote.

EVANS, H.M., and SWEZY, O. (1929): Mem. Univ. Calif., 9, 1.

FAIERMAN, E. (1960): Arch. Dis. Childh., 35, 285.

FELDMAN, H.A. (1958): Pediatrics, 22, 559.

FORD, C.E. (1959): Report to Conference on Human Chromosomal Abnormalities. Staples Press, London.

FORD, C.E. (1961): Methodology in Human Genetics. Ed. W.J. Burdette, Columbia University Press, New York.

FORD, C.E. (1962): Chromosomes in Medicine. J.L. Hamerton, Little Club Clinic Dev. Med. 5 National Spastics Society and Heinemann Medical Books, London.

FORD, C.E., and HAMERTON, J.L. (1956a): Stain Technol., 31, 247.

FORD, C.E., and HAMERTON, J.L. (1956b): Nature, 178, 1020.

FORD, C.E., JACOBS, P.A., and LAJTHA, L.G. (1958): Nature, 181, 1565.

FORD, C.E., JONES, K.W., POLANI, P.E., De ALMEIDA, J.C., and BRIGGS, J.H. (1959a): Lancet, 1, 711.

FORD, C.E., POLANI, P.E., BRIGGS, J.H., and BISHOP, P.M.F. (1959b): Nature, Lond., 183, 1030.

FRACCARO, M. (1960): Int. J. rod. Biol., Suppl., 117.

FRACCARO, M. (1962): Chromosomes in Medicine. Ed. J.L. Hamerton. Little Club Clinic Dev. Med. 5 National Spastics Soc. and Heinemann, London.

FRACCARO, M., and LINDSTEN, J. (1960): Lancet, 2, 1303.

FRACCARO, M., IKKOS, D., LINDSTEN, J., LUFT, R., and KAIJSER, K. (1960): Lancet, 2, 1144.

FRASER, F.C. (1952): Canad. M. A. J., 66, 258.

FRASER, F.C. (1954): J. Pediat., 44, 85.

FRASER, F.C. (1959): J. Chr. Dis., 10, 97.

FRIZZI, G. (1948): Ric. Sci., Mem., 18, 119.

GARROD, A.E. (1923): Inborn Errors of Metabolism, 2nd. ed. Frowde, Hodder & Stoughton, London.

GALLAGHER, J.P., and MALONE, R.G.S. (1956): J. Obstet. Gynaec. Brit. Emp., 63, 287.

GEISSLER, A. (1889): Z. K. Sachs. Statist. Bureau, 35, 1.

GERMAN, J., RANKIN, J.K., HARRISON, P.A., DONOVAN, D.J., HOGAN, W.J., and BEARN, A.G. (1962): J. Pediat., 60, 503.

GIACOMINI, C. (1882 - 1891): Quoted by Mall and Meyer (1921).

GILBERT, C.W., MULDAL, S., LAJTHA, L.G., and ROWLEY, J. (1962): Nature, Lond., 195, 869.

GRAHAM, M.A., and BARR, M.L. (1952): Anat. Rec., 112, 709.

GRAHAM, M.A. (1954): Anat. Rec., 4, 469.

GRANVILLE, A.B. (1834): Graphic Illustration of Abortion and the Diseases of Menstruation. London.

GREENHILL, J.P. (1957): Amer. J. Obstet. Gynec., 73, 576.

GREGG, N.M. (1941): Trans. Ophthal. Soc. Aust., 3, 35.

GREY, J.D. (1956): Amer. J. Obstet. Gynec., 72, 615.

GREY, J.D. (1957): Amer. J. Obstet. Gynec., 74, 111.

GREY, J.D., TUPPER, C., and ROWSE, J.A. (1959): Am. J. Obstet. Gynec., 78, 325.

GROSSER, O. (1927): Fruhentwicklung. Eihautbildung und Placentation der Menschen und der Saugettierre. Munich, Bergmann.

GRUENWALD, P. (1958): Amer. J. Path., 34, 77.

GRUENWALD, P., and MAYBERGER, H.W. (1960): Arch. Path., 70, 687.

GRUMBACH, M.M., MORISHINAM, A., and CHU, E.H.Y. (1960): Amer. J. Dis. Child., 100, 548.

GUNZ, F.W., FITZGERALD, P.H., and ADAMS, A. (1962): Brit. Med. J., 2, 1097.

GUTHKELCH, A.N. (1962): Brit. J. prev. soc. med., 16, 159.

HAAS, H. (1906): Beitrage zur Geburtshilfe, 10, 483.

HALSEY, H., and DOUGLAS, R. (1957): Surg. Clin. North America, April, 421.

HAMILTON, W.J., and BOYD, J.D. (1960): J. Anat. (Lond.), 94, 297.

HAMILTON, W.J., BOYD, J.D., and MOSSMAN, H.W. (1962): Human Embryology. Heffer & Sons Ltd., Cambridge.

HANSEMANN, D. (1891): Virchow's Arch., 123, 356.

HARNDEN, D.G. (1960): Brit. J. exp. Path., 41, 31.

HARVEY, W. (1651): Excercises on the generation of Animals.

HATHOUT, H.M. (1963): J. Obstet. Gynaec. Brit. Comwlth., 70, 859.

HAYMAN, D.J. (1961): Brit. med. J., 2, 1499.

HAYWARD, M.D., and BOWER, B.D. (1960): Lancet, 2, 844.

HAYWARD, M.D., and CAMERON, A.H. (1961): Lancet, 2, 623.

HEINRICHS, E.H., and ALLEN, S.W. (1963): Clin. Pediat., 2, 25.

HERTIG, A.T. (1960): The Placenta and Fetal Membranes. Ed. C. Villee. Williams & Wilkins, Baltimore, 109.

HERTIG, A.T., and EDMONDS, H.W. (1940): Arch. Path., 30, 260.

HERTIG, A.T., and MANSELL, H. (1956): Atlas of Tumor Pathology. Armed Forces Institute of Pathology. Washington D. C.

HERTIG, A.T. and ROCK, J. (1949): Amer. J. Obstet. Gynec., 58, 968.

HERTIG, A.T., and SHELDON, W.H. (1943): Ann. Surg., 117, 596.

HEUSER, A. (1958): Gynak. u. Geburtsh. Bern (Mai).

HICKS, S.P. (1952): J. Pediat., 40, 489.

HIENZ, H.A. (1961): Geburtsh. u. Frauenh., 21, 144.

HILL, A.B., DOLL, R., GALLOWAY, T., and HUGHES, J.P.W. (1958): Brit. J. Prev. & Social Med., 12, 1.

HIS, W. (1880): Anatomic menschlicher Embryonen. F. Vogel, Leipzig.

HIS, W. (1882, 1892): Quoted by Mall and Meyer (1921).

HITSCHMANN, F., and ADLER, L. (1908): Monatsschr. f. Geburtsh. u. Gynakol., 27, 182.

HOBBS, J.E., and PRICE, C.N. (1940): Amer. J. Obstet. Gynec., 39, 39.

HOET, J.P., HOET, J.J., GOMMERS, A. (1959): Pro. Roy. Soc. Med., 52, 813.

HSU, T.C. (1959): 16th Growth Symposium: Developmental Cytology. Ed. Dorothea Rudnick. Ronald Press.

HUBER, C.P., MELIN, J.R., and VELLIOS, F. (1957): Amer. J. Obstet. Gynec., 73, 569.

HUNTER, W.F., and LENNOX, B. (1954): Lancet, 2, 633.

HYRTL, J. (1870): Die Blutgefasse der Menschlichen Nachgeburt in normalen und abnormen Verhaltnissen. W. Braumuller, Vienna.

ILBERY, P.L.T., LEE, C.W.G., and WINN, S.M. (1961): Med. J. Aust., 2, 182.

INGALLS, T.H. (1950): New Eng. J. Med., 243, 67.

INGALLS, T.H. (1960): Ciba Foundation Symposium on Congenital Malformations. Churchill. London, 51.

ISHIZAKI, Y. (1960): Obst. & Gynec., 15, 528.

JACOBS, P.A., and STRONG, J.S. (1959): Nature, Lond., 183, 302.

JACOBS, P.A., BAIKIE, A.G., COURT BROWN, W.M., MacGREGOR, T.N., MacLEAN, N., and HARNDEN, D.G. (1959a): Lancet, 2, 423.

JACOBS, P.A., FORREST, H., ROY, J.R., STEWART, J.S.S., and LENNOX, B. (1959b): Lancet, 2, 591.

JACOBS, P.A., HARNDEN, D.G., COURT BROWN, W.M., GOLDSTEIN, J., CLOSE, H.G., MacGREGOR, T.N., MacLEAN, N., and STRONG, J.A. (1960): Lancet, 1, 1213.

JACOBS, P.A., BUCKTON, K.E., COURT BROWN, W.M., KING, M., McBRIDE, J.A., MacGREGOR, T.N., and MacLEAN, N. (1961a): Lancet, 1, 1183.

JACOBS, P.A., BUCKTON, K.E., COURT BROWN, W.M., KING, M., McBRIDE, J.A., MacGREGOR, T.N., and MacLEAN, N. (1961b): Lancet, 1, 1183.

JACOBS, P.A., BRUNTON, M., COURT BROWN, W.M., DOLL, R., and GOLDSTEIN, H. (1963): Nature, Lond., 197, 1080.

JACOBS, P.A., BRUNTON, M., and COURT BROWN, W.M. (1964): In press. Ann. hum. genet.

JAVERT, C.T. (1957): Spontaneous and Habitual Abortion. McGraw-Hill, New York, 13.

JAVERT, C.T., and BARTON, B. (1952): Amer. J. Obst. & Gynec., 63, 1065.

JAVERT, C.T., FINN, W.F., and STANDER, H.J. (1949): Amer. J. Obst. & Gynec., 57, 878.

JEFFCOATE, T.N.A. (1963): Principles of Gynaecology. 2nd ed., Butterworths.

KAJIL, T., SHINOHARA, M., KIKUCHI, K., DOHMEN, S., and AKICHIKA, M. (1963): Lancet, 2, 889.

KAUFMANN, E., and STAEMMLER, M. (1957): Lehrbuch der Speziellen Pathologischen Anatomie. Berlin, 2, 340.

KELLER, R., and ADRIAN, J. (1938): Gynec. et obst., 38, 332.

KLATER, H., and WARKANY, J. (1959): Physiol. Rev., 39, 69.

KLINGER, H.P. (1957): Acta. anat. (Basel), 30, 371.

KLINGER, H.P., and LUDWIG, K.S. (1957): Z. Anat. Entw. Gesch., 120, 95.

KLINGER, H.P., and SCHWARZACHER, H.G. (1962): Cytogenetics (Basel), 1, 266.

KODANI, M. (1957): Proc. Nat. Acad. Sci, 43, 285.

KOSIN, I.L., and ISHIZAKI, H. (1959): Science, 130, 43.

KRONE, Von H.A. (1962): Geburtshilf. Frauenheilk, 22, 1294.

LANCET (1960): 1, 1063.

LANCET (1961): 2, 31.

LANCET (1962): 2, 931.

LANCET (1963): 1, 705.

LEJEUNE, J., TURPIN, R., and GAUTIER, M. (1959a): Ann. de Genet., 1, 41.

LEJEUNE, J., GAUTHIER, M., and TURPIN, R. (1959b): C.R. Acad. Sci. (Par.), 248, 602.

LEJEUNE, J., TURPIN, R., and DECOURT, J. (1960): C.R. Acad. Sci. (Par.), 250, 2468.

LENNOX, B. (1960): Recent Advances in Pathology. 7th ed. Editor C.V. Harrison. Churchill, Lond., 275.

LENZ, W. (1962): Lancet, 1, 45.

LEWIS, A.J. (1962): Lancet, i, 866.

LEWIS, T.L.T. (1964): Progress in Clinical Obstetrics and Gynaecology. 2nd ed., Churchill, London.

LITTLE, W. (1960): Obst. & Gynec., 15, 109.

LITTLE, W. (1961): Obst. & Gynec., 17, 695.

LOESCHCKE, H. (1948): Virchows Arch. path. Anat., 315, 499.

LOVE, W.C., and BUCKLIN, R. (1958): Obst. & Gynec., 11, 459.

LUDWIG, W., and BOOST, Ch. (1940): Z. Naturwiss, 94, 1.

LUNDSTROM, R. (1962): Acta paediat. Uppsala, Supp. 133.

LYON, F. (1960): Obst. & Gynec., 16, 719.

LYON, M.F. (1961): Nature (Lond.), 190, 372.

MACLEAN, N., et al. (1962): Lancet, 1, 293.

MacMAHON, B., HERTIG, A., and INGALLS, T. (1954): Obst. & Gynec., 4, 477.

MALL, F.P. (1900): A contribution to the study of the pathology of early human embryos. Johns Hopkins Hosp. Reports, vol. 9.

MALL, F.P. (1903): Second contribution to the study of the pathology of early human embryos. Contributions to Medical Research, dedicated to Victor Clarence Vaughan. Ann Arbor, Michigan.

MALL, F.P. (1904): Pathological human embryos. Wood's Ref. Handbook Med. Sci. New York.

MALL, F.P. (1908): J. Morphol., 19, 1.

MALL, F.P., and MEYER, A.W. (1921): Studies on Abortions: A Survey of Pathologic Ova in the Carnegie Embryological Collection. Contributions to Embryology (No. 56), Carnegie Institution of Washington, Publication No. 275.

MANSON, M., LOGAN, W.P.D., and LOY, R.M. (1960): Rubella and Other Virus Infections during Pregnancy. Rep. Publ. Health Med. Subjects No. 101, H.M.S.O., London.

McBRIDE, W.G. (1961): Lancet, 2, 1358.

MENDEL, G. (1886): Naturforsch. ver. Brumn, 4, 3.

MILDENSTEIN, F., MASSENBACH, W., and RUTHER, K. (1964): Geburtsh. Frauenheilk., 24, 1.

MINOT, C. (1892): Human Embryology. Wood & Co., New York, 363.

MONTGOMERY, T.L. (1936): Amer. J. Obstet. Gynec., 31, 253.

MOORE, K.L., and BARR, M.L. (1954): Acta Anat., 21, 197.

MOORE, K.L., and BARR, M.L. (1955): Lancet, ii, 57.

MOORE, K.L., and BARR, M.L. (1957): Brit. J. Canc., 11, 384.

MOORE, C.R., and PRICE, D. (1948): J. exp. Zool., 108, 171.

MOORE, K.L., and HAY, J.C. (1963): Canad. Med. Ass. J., 88, 1077.

MOOREHEAD, P.S., NOWELL, P.C., MELLMAN, W.J., BATIPPS, D.M., and HUNGERFORD, D.A. (1960): Exp. Cell. Res., 20, 613.

MORISON, J.E. (1963): Foetal and Neonatal Pathology. Butterworths, London, 14.

MULDAL, S. (1962): Lancet, 2, 1384.

MULDAL, S., and OCKEY, C.H. (1960): Lancet, 2, 492.

MULLER, H. (1847): Quoted by Mall and Meyer (1921).

MULLER, H.J. (1932): Proceedings of the Sixth International Congress of Genetics, vol. 1, 213.

MURPHY, D.P. (1947): Congenital Malformations, 2nd ed., Lippincott, Philadephia.

NEEL, J.V., and SCHULL, W.J. (1956): The Effects of Exposure to the Atomic Bombs on Pregnancy Terminations in Hiroshima and Nagasaki. Nat. Acad. Sci., Nat. Res. Counc. Publ. No. 461, Washington D.C.

NILSSON, L. (1957): Acta obstet. gynec. scand., 35, Suppl. 7.

NOVAK, E., and NOVAK, E.R. (1958): Gynecologic and Obstetric Pathology. 4th ed., Saunders & Co., Philadelphia, 592.

NOWELL, P.C., and HUNGERFORD, D.A. (1960): Science, 132, 1497.

Obstetrical and Gynaecological Survey (1961): 16, 345.

OHNO, S. (1963): Acta Cytologica, 7 (3), 147.

OHNO, S., and HAUSCHKA, T.S. (1960): Cancer Res., 20, 541.

OTTO, W. (1830): Lehrbuch der Pathologischen Anatomie.

PAGET, J. (1882): Lancet, 2, 1017.

PAINTER, T.S. (1921): Science, 53, 503.

PAINTER, T.S. (1923): J. Exp. Zool., 37, 291.

PANUM, P.L. (1860): Quoted by Mall and Meyer (1921).

PARMELEE, A.H. (1931): Amer. J. Dis. Child., 42, 1155.

PATAU, K., SMITH, D.W., THERMAN, E., INHORN, S.L., and WAGNER, H.P. (1960): Lancet, 1, 790.

PARK, W.W. (1958): J. Path. Bact., 75, 257.

PARK, W.W. (1959): Modern Trends in Pathology. Butterworth. London. 186.

PATZELT, V. (1956): Z. Zellforsch. u. mikroscop. Anat., 43, 644.

PAUL, J. (1960): Cell and Tissue Culture. 2nd ed., Livingstone.

PECKHAM, C.H. (1936): Surg. Gynec. & Obst., 63, 109.

PENROSE, L.S. (1957): J. Ment. Defic. Res., 1, 4.

PENROSE, L.S., and DELHANTY, J.D.A. (1961): Lancet, 1, 1261.

PFAUNDLER, M. (1936): Z. Kinderhk., 57, 185.

PFEIFFER, R.A., and KOSENOW, W. (1962): Lancet, 1, 45.

PIRAUX, P. (1958): Bruxelles Med., 38, 169.

PLEYDELL, M.J. (1960): Brit. med. J., 1, 309.

PLIESS, G. (1962): Lancet, 1, 1128.

POLANI, P.E. (1962): Chromosomes in Medicine. Ed. J.L. Hamerton, Little Club Clinic Dev. Med. 5, National Spastics Soc. and Heinemann Medical Books, London.

POTTER, E.L. (1961): Pathology of the Fetus and Infant. Year Book Medical Publishers, Chicago, 72.

POWELL, P.D., and JOHNSTONE, J.M. (1962): Brit. med. J., 2, 1327.

RECORD, R.G. (1956): Brit. J. prev. soc. Med., 10, 19.

REED, T.E., SIMPSON, N.E., and CHOWN, B. (1963): Lancet, 2, 467.

ROBLES, R. and PRATT, J. (1961): Obst. & Gynec., 17, 378.

ROSA, P.A., and FARNARD, A.E. (1951): Int. J. Sexol., 4, 160.

ROWLEY, J., MULDAL, S., GILBERT, C.W., LAJTHA, L.G., LINDSTEN, J., FRACCARO, M., and KAIJSER, K. (1963): Nature, Lond., 197, 251.

RUGH, R., and GRUPP, E. (1960): Amer. J. Roentgen., 84, 125.

RUSSEL, C.S. (1963): Modern Trends in Obstetrics (3). Ed. R.J. Kellar, Butterworths, London. 35.

RUSSEL, S.C., and McKICHAN, M.D. (1962): Lancet, 1, 429.

RUTHER, H. (1939): Arch. Gynak., 168, 44.

RUYSCH, F. (1691): Observ. Anat. Chir. Centuria. Amstelod.

SACHS, L., and DANON, M. (1956): Genetica, 28, 201.

SANDERSON, A.R., and STEWART, J.S.S. (1961): Brit. med. J., 2, 1065.

SALVAGGIO, A.T., NIGOGOSYAN, G., and MACK, H.C. (1960): Amer. J. Obstet. Gynec., 80, 1013.

SAVAGE, J.E. (1957): Amer. J. Obstet. Gynec., 73, 577.

SCHAEFER, G. (1939): Amer. J. Obstet. Gynec., 38, 1066.

SCHATZ, F. (1900): Arch. f. gynak., 60, 81.

SCHAUTA, F. (1881): Arch, Gynak., 17, 19.

SCHMID, W. (1962): Cytogenetics, 1, 199.

SCHMORL, G. (1893): Pathologische - anatomische Untersuchungen uber Puerperal Eklampsie. Vogel. Leipzig.

SCOTT, J.S. (1960): J. Obstet. Gynaec. Brit. Emp., 67, 904.

SCOTT, J.S. (1963): Modern Trends in Obstetrics. Ed. R.J. Kellar, Butterworths, London.

SEXTON, L.I., HERTIG, A.T., REID, D.F., KELLOGG, F.S., and PATTERSON, W.S. (1950): Amer. J. Obstet. Gynec. 59, 13.

SHETTLES, L.B. (1961): Obst. & Gynec., 18, 122.

SIDALL, R.S., and HARTMAN, F.W. (1926): Amer. J. Obstet. Gynec., 12, 683.

SILBERNAGEL, W.M., and FIDDLER, R.S. (1942): Amer. J. Obstet. Gynec., 44, 712.

Simpson Memorial Maternity Pavilion, Royal Infirmary, Edinburgh (1962): Medical and Clinical Report.

SINCLAIR, J.G. (1948): Anat. Rec., 102, 245.

SLATIS, H.M. (1953): Amer. J. hum. genet., 5, 21.

SLIFER, E.H. (1934): J. Exp. Zool., 67, 137.

SMITH, D.W., PATAU, K., THERMAN, E., and INHORN, S.L. (1960): J. Pediat., 57, 338.

SMITH, S.G. (1945): Sci. Agric., 25, 566.

De SNOO (1932): Z. Gynak., 56, 2833.

SNYDER, L.H., and DAVID, P.R. (1953): Clinical Genetics. Ed. A. Sorsby. Butterworth, London.

SOLTH, K. (1962): Obstet. & Gynec. Survey, 17, 640.

SPANNER, R. (1956): Translated by Harris, B.A., Amer. J. Obstet. Gynec., 71, 350.

SPECK, G., and PALMER, R.E. (1961): Southern Med. J., 54, 48.

SPEIRS, A.L. (1962): Lancet, 1, 303.

SPIVACK, M. (1941): Amer. J. Obstet. Gynec., 52, 387.

STABLER, F. (1962): Lancet, 1, 591.

STERN, C. (1959): J.M. Educ., 34, 301.

STEVENSON, A.C. (1959): Ann. hum. genet., 23, 415.

STEVENSON, A.C. (1960): Ciba Foundation Symposium on Congenital Malformations. Churchill, London, 241.

STEVENSON, A.C. (1961): Proc. roy. Soc. Med., 54, 893.

STEVENSON, A.C., and McCLARIN, R.H. (1957): Nature (Lond.), 180, 198.

STRAUCH, H. (1938): Zentralb. f. Gynak., 62, 1371.

SULLIVAN, R.Y. (1922): Amer. J. Obstet. & Gynec., 3, 520.

SUTER, M., and WICHSER, C. (1948): Amer. J. Obstet. Gynec., 55, 489.

SWAN, C. (1949): J. Obstet. Gynaec. Brit. Emp., 56, 341, 591.

SWAN, C., TOSTEVIN, A.L., and BLACK, G.H.B. (1946): M. J. Australia, 2, 889.

SZONTAGH, F.E., JAKOBOVITIS, A., and MEHES, Ch. (1961): Nature, 192, 476.

THAISZ, K. (1938): Zentralb. f. Gynak., 62, 1937.

THOMAS, J. (1963): Arch. Gynak., 198, 216.

THOMAS, J., and SCHWEIGERT, M. (1956): Gelurtsh. u. Frauenh., 16, 1031.

THOMAS, L. (1961): in Congenital Malformations (1st Inter. Conf.), Lippincott, Philadelphia and Montreal, 230.

TIETZE, C. (1953): Pregnancy Wastage. Ed. Engle, E.T., C.C. Thomas, Springfield, Ill., 135.

TJIO, J.H., and LEVAN, A. (1956): Hereditas, Lund., 42, 1.

TJIO, J.H., and PUCK, T.T. (1958a): J. exp. Med., 108, 259.

TJIO, J.H., and PUCK, T.T. (1958b): Proc. Nat. Read. Sci., 44, 1229.

TOUGH, I.M., COURT BROWN, W.M., BAIKIE, A.G., BUCKTON, K.E., HARNDEN, D.G., JACOBS, P.A., KING, M., and McBRIDE, J.A. (1961): Lancet, 1, 411.

TRICOMI, V., SERR, D., and SOLISH, G. (1960): Amer. J. Obstet. Gynec., 79, 504.

TURNBULL, E.P.N., and WALKER, J. (1956): J. Obstet. Gynaec. Brit. Emp., 63, 553.

TURPIN, R., LEJEUNE, J., LAFOUCADE, J., and GAUTIER, M. (1959): C.R. Acad. Sci. (Par.), 248, 3636.

UCHIDA, I.A., BOWMAN, J.M., and WANG, H.C. (1962): New Engl. J. Med., 266, 1198.

United Nations (1949 - 50): Demographic Year Book. United Nations, New York, 117.

United Nations Scientific Committee (1958): Effects of Atomic radiation. Official records: 13th session Suppl. No. 17(A/3838). New York.

United Nations Scientific Committee (1962): Effects of atomic radiation. Official records: 17th session. Suppl. No. 16 (A/5216). United Nations, New York.

VALAORAS, V.G. (1953): Pregnancy Wastage. Ed. Engle, E.T., C.C. Thomas, Springfield, Ill., 143.

VEIT, J. (1905): Die Verschleppung der Chorionzotten (Zottendeportation). Ein Beitrag zur geburtshilflichen Physiologie und Pathologie. Bergmann, Wiesbaden.

WADDINGTON, H.K. (1956): Obst. & Gynec., 7, 319.

WAGNER, D. (1958): Geburtsh. u. Frauenheilk., 18, 1460.

WAIDL, E. (1960): Zbl. f. Gynak., 82, 1902.

WARKANY, J. (1959): J. Chr. Dis., 10, 84.

WARKANY, J. (1960): Ciba Foundation Symposium on Congenital Malformations. Churchill.. London.

WARKANY, J. (1964): Arch. Path., 75, 579.

WEBER, J. (1963): Acta Obstet. Gynec. Scand., 42, 259.

WELSHONS, W.J., and RUSSEL, L.B. (1959): Proc. u. s. Nat. Acad. Sci., 45, 560.

WESTERMARK, H. (1925): Inaug. Diss. Stockholm. Quoted from Morison (1963).

WICHRAMASURIYA, G.A. (1935): J. Obstet. Gynaec. Brit. Emp., 42, 816.

WIDHOLM, O., MEYER, B., and NUMERS, C. (1963): Gynaecologia, 155, 386.

WIEDEMANN, H.R. (1961): Med. Welt., 37, 1863.

WIEMAN, H.L. (1917): Am. J. Anat., 21, 1.

WIGGLESWORTH, J.S. (1962): J. Obstet. Gynaec. Brit. Comwlth., 69, 355.

WILLIAMS, J.W. (1900): Amer. J. Obstet., 41, 775.

WILLIAMS, J.W. (1931): Obstetrics. D. Appleton Century, New York.

WILLIAMS, W. (1927): Amer. J. Obstet. Gynec., 13, 1.

WILKINS, L. (1960): J. Amer. Med. Ass., 172, 1028.

WILSON, J.G. (1959): J. chr. Dis., 10, 111.

WILSON, K.W. (1926): Contr. Embryol. Carneg. Instn., 18 (91), 23.

WIRTINGER, W. (1941): Wien. klin. Wchnschr., 54, 139.

WISLOCKI, I.B., and BENNETT, H.S. (1943): Amer. J. Anat., 73, 335.

WOODRUFF, M.F.A. (1958): Proc. roy. Soc. B., 148, 68.

WOODRUFF, M.F.A., and LENNOX, B. (1959): Lancet, 2, 467.

WOOLLAM, D.H.M. (1962): Brit. Med. J., 2, 236.

World Health Organisation (1950): Subcommittee on the Definition of Stillbirth and Abortion: Report on the first session. World Health Organisation Technical Report Series No. 25, 9 - 16.

YOUNG, J. (1914): J. Obstet. Gynaec. Brit. Emp., 26, 1.

YOUNG, M., and MARTIN, J.D. (1963): British Obstetric and Gynaecological Practice. Obstetrics. W. Heinemann, Ltd., London, 49.

YOUNG, W.C. (1953): Pregnancy Wastage. Engle, E.T., C.C. Thomas, Springfield, Ill., 38.

ZAWISCH, C. (1955): Z. Zellforsch. u. mikroskop. Anat., 42, 94.

ZEEK, P.M., and ASSALI, N.S. (1952): Amer. J. Obstet. Gynec., 64, 1191.

ZELLWEGER, H., MIKAMO, K., and ABBO, G. (1962): Helv. Paediat. Acta, 17, 290.

ATLAS OF ILLUSTRATIONS.

Fig. I

Blighted ovum of the empty sac variety. The specimen was received intact, and when opened there was no trace of embryo or cord. Histological examination showed hydatidiform degeneration.

Fig. 2

Another example of a blighted ovum of the empty sac variety. Histological examination showed total avascularity of the villi.

Fig. I

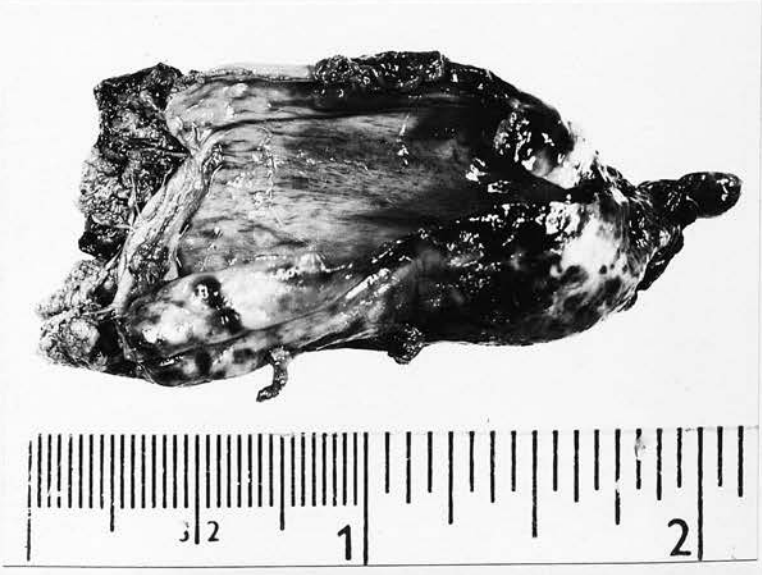

Fig. 2

Fig. 3, a

Nodular embryo.

Fig. 3, b

A close-up view of the same embryo shown in fig. 3, a.

Fig. 3, a

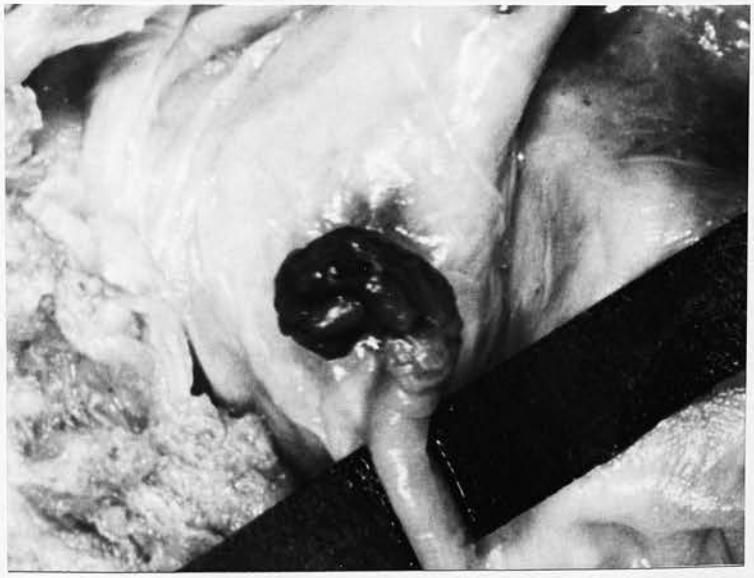

Fig. 3, b

Fig. 4, a

Nodular embryo.

Fig. 4, b

A close-up view of the same embryo shown in Fig. 4, a. The umbilical vessels are clearly seen at the foetal end of the cord. Although the cord contained the normal three vessels, histological examination showed very poor vascularity of the villi.

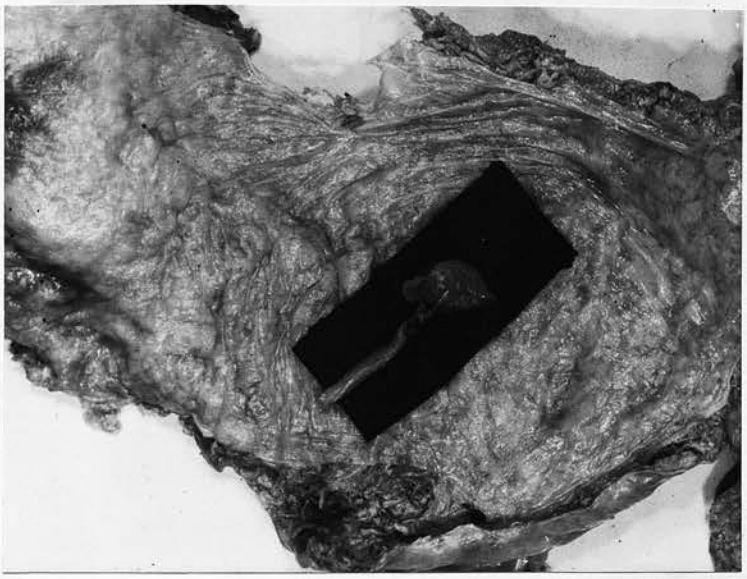

Fig. 4, a

Fig. 4, b

Fig. 5

Mono-ovular twin pregnancy. In the upper left quadrant of the photograph there is a nodular embryo that has no umbilical cord (note the black pointer). The larger twin has an abnormally thin umbilical cord.

Fig. 6

Cylindric embryo. A cephalic end could be recognized (more so under the dissecting microscope), but the rest of the bodymwas a featureless mass. A cystic formation is seen along the umbilical cord.

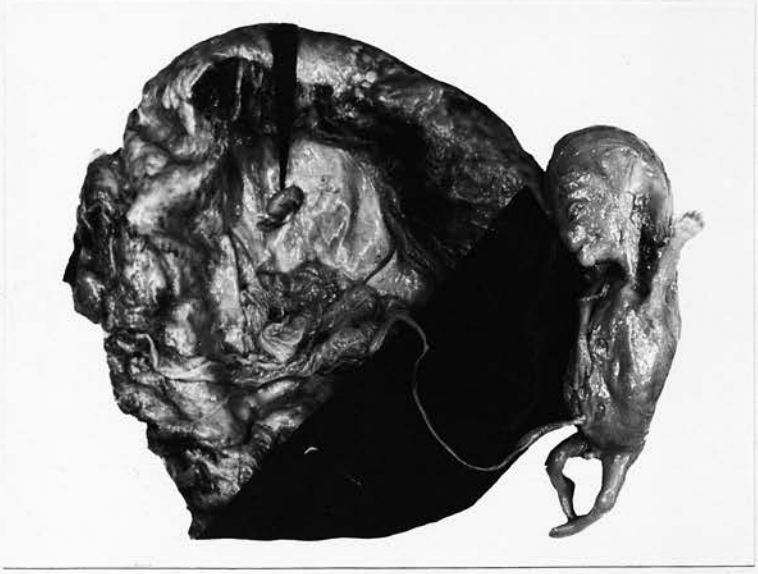

Fig. 5

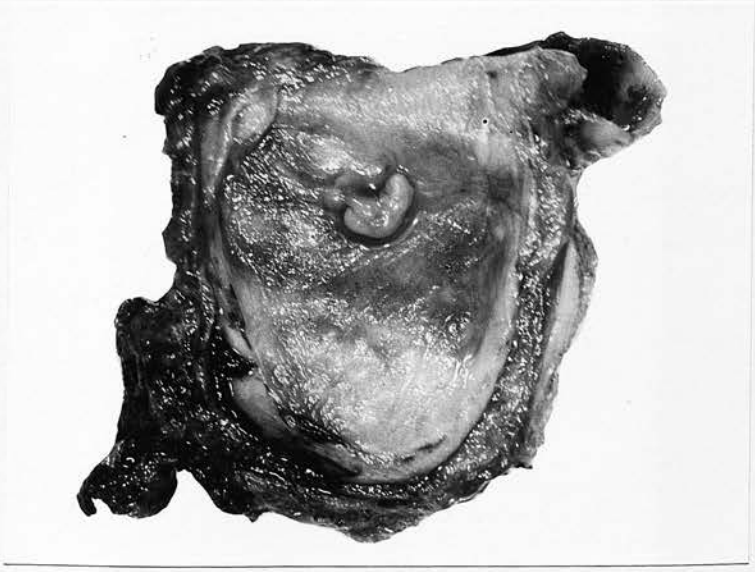

Fig. 6

Fig. 7

Stunted embryo. The CR length was 8 mm. Although the embryo was traumatized, the cephalic end, trunk and limb buds were recognizable, From a case of spontaneous abortion at IO weeks. The patient was a habitual aborter and this was her fourth abortion in succession.

Fig. 8

A normal embryo in an intagt amniotic sac. From a case of spontaneous abortion at 8 weeks,

Fig. 7

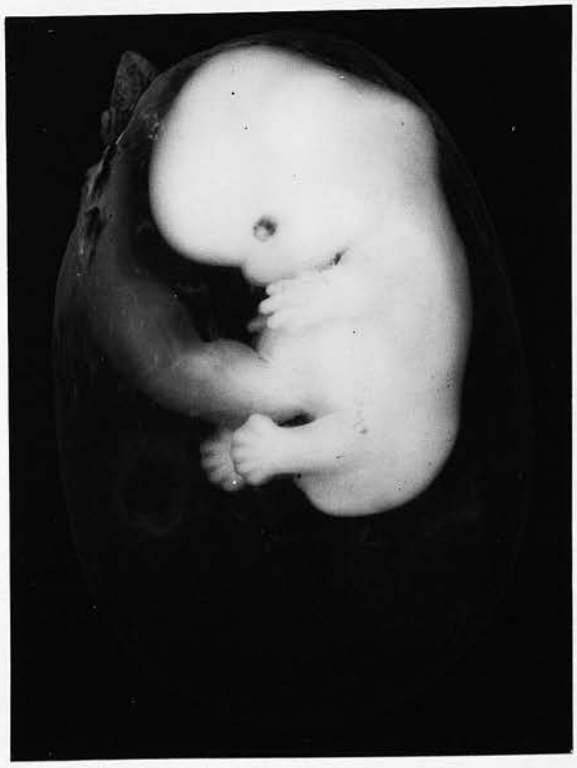

Fig. 8

Fig. 9

Imperforate anus. Female foetus aborted spontaneously at 26 weeks. The pregnancy was illigitimate but the mother, I6 years old, married a short while before abortion. Early in this pregnancy (6-8 weeks), she had acute rheumatic fever.

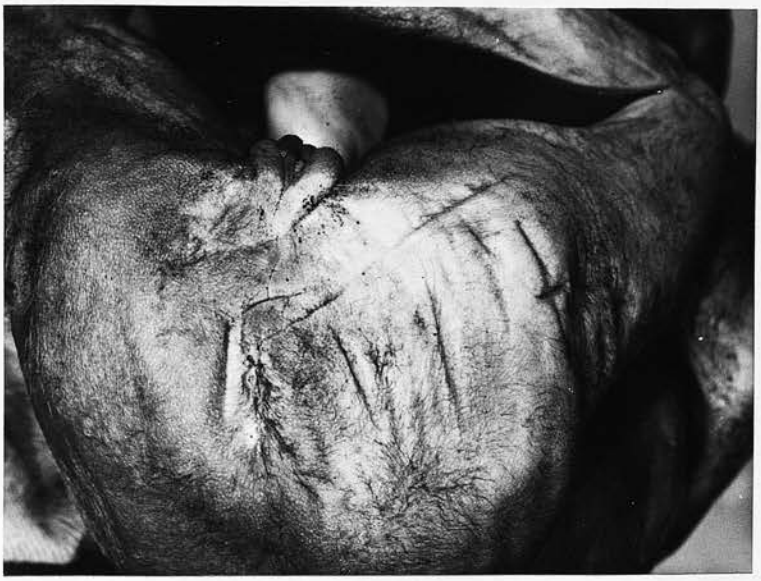

Fig. 9

Fig. IO

Large umbilical hernia. There is also abnormal torsion of the umbilical cord. From a case of missed abortion completed at 20 weeks.

Fig. II

Umbilical hernia. The sac has been slit open, exposing loops of small intestines.

Fig. IO

Fig. II

Fig. I2

Exomphalos. The loops of intestine are shining through the covering peritoneum at the base of the umbilical cord.

Fig. I3

Exomphalos. The foetus was aborted at I5 weeks, and had also abnormal pulmonary lobulation in the form of a bilobed right lung.

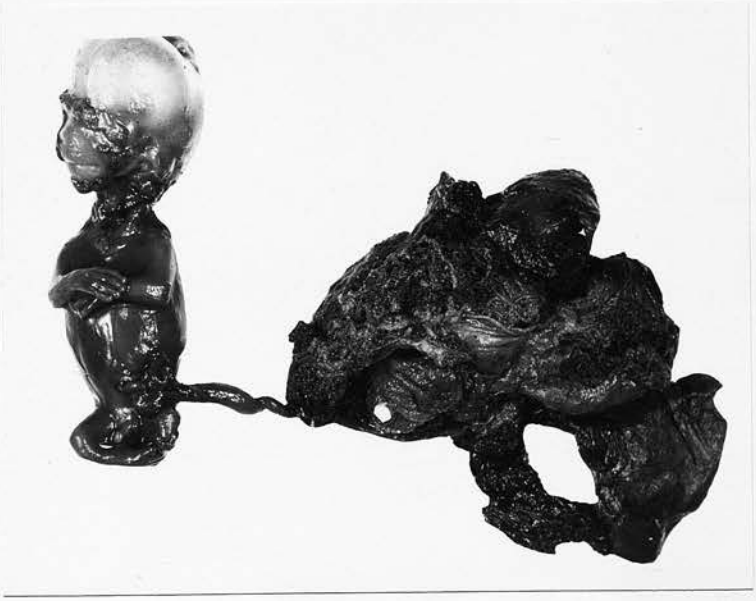

Fig. I2

Fig. I3

Fig. I4

Meckel's diverticulum. Case of illigitimate pregnancy ending in spontaneous abortion at 24 weeks. The foetus had a gap in the skull vault, bilobed right lung, polydactyly, accessory splenic tissue, as well as gastro-intestinal, cardio-vascular and renal abnormalities.
(See Figs. I5, 22, 28 & 4I)

Fig. I5

A tumour arising from the first part of the duodenum. The tumour was firm, and on section contained no cavity. Histologically, the tumour contained pancreatic tissue and was reported to be of the nature of a hamartoma.
(Histological report by Dr.Hunt of the Department of Pathology, University of Edinburgh).

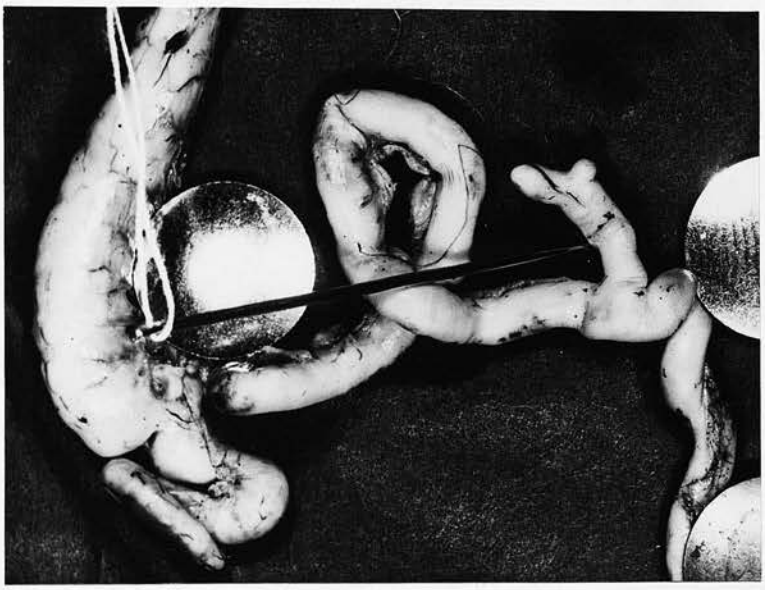

Fig. I4

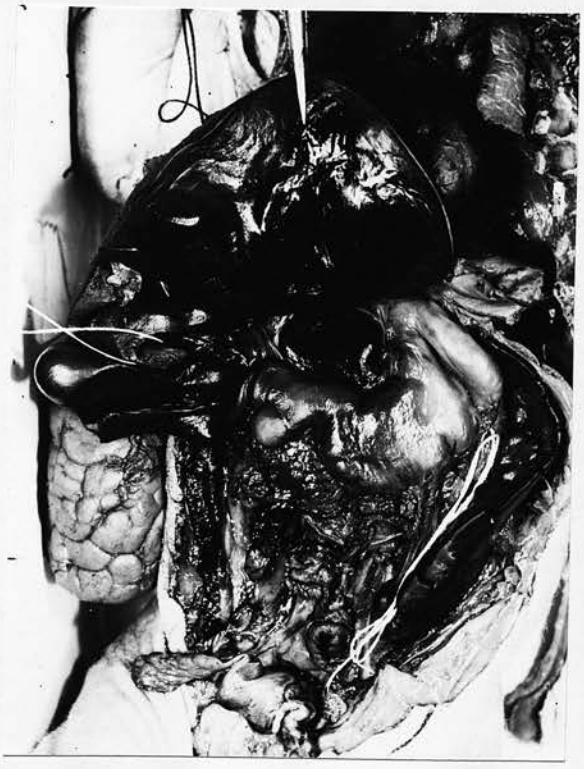

Fig. I5

Fig. I6

The mass arising from the outer wall of the ileum was described macroscopically as a 'para-ileal' tumour. The tumour contained a cavity but communication with the lumen of the intestine could not be established. Histologically it was lined with small-intestinal mucous membrane. It might represent a poor attempt at reduplication. From a case of illigitimate twin pregnancy ending at I5 weeks. The patient and her fiance each was a twin.

Fig. I7

Persistent mesentry of the descending colon in a nonmacerated foetus aborted spontaneously at 20 weeks. The mother was a grande multipara. Earlier in this pregnancy (8 weeks) she attempted abortion by taking some sort of tablets but without success. There was no other abnormality in the foetus.
According to Arey (I954), fusion of this part of the mesentry should have been complete by the end of the fifth month. He points out that this fusion is not the result of nearness and pressure of the adjacent peritoneal surfaces, but it represents the carrying out of a definite hereditary pattern.

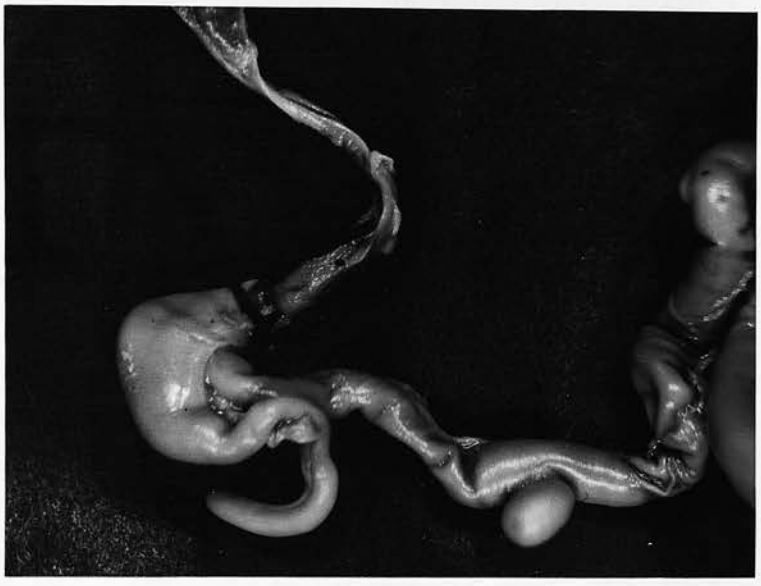

Fig. I6

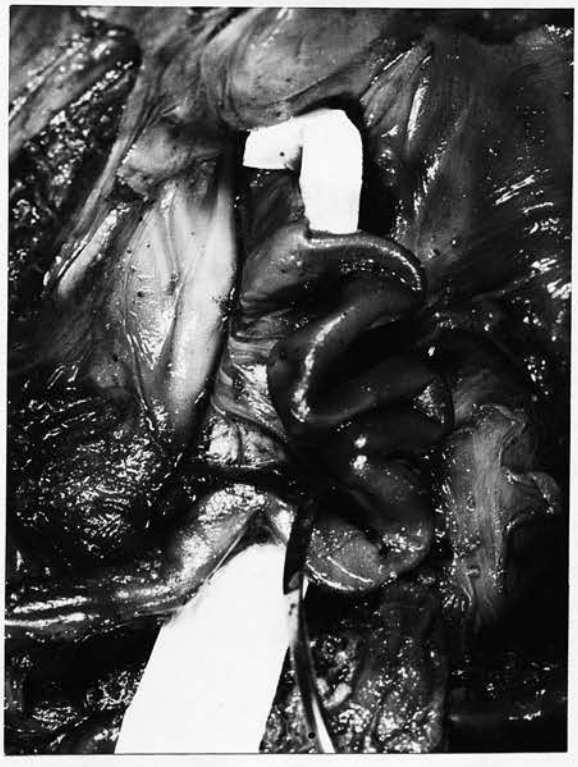

Fig. I7

Fig. I8

Fenestration of the lesser omentum. A cirgular defect in the lesser omentum renders the caudate lobe of the liver directly accessible above the duodenum.

Fig. I9

Volvulus of the small intestines.

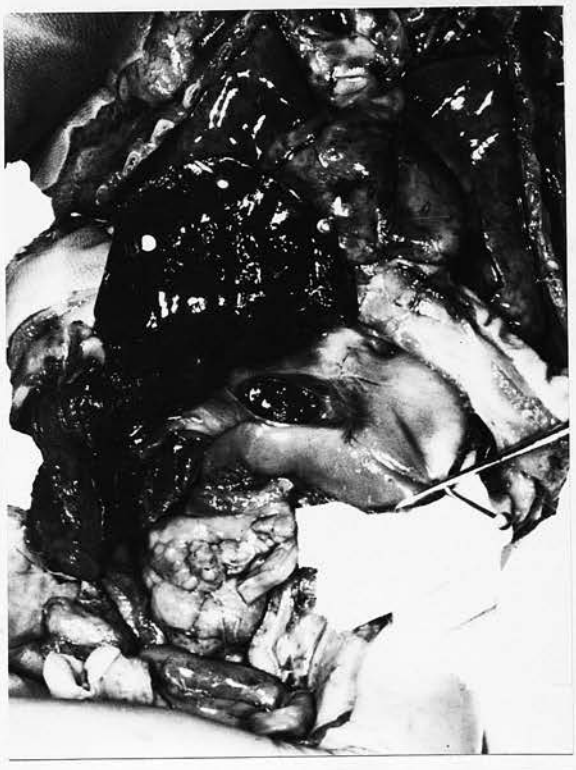

Fig. I8

Fig. I9

Fig. 20, a

Volvulus of the small intestine of a 19 week foetus. Note the congestion and distension of the affected segment in contrast to the normal intestine.

Fig. 20, b

A profile view of the same specimen.

Fig. 20, a

Fig. 20, b

Fig. 2I

Meconium obstruction with perforation of the small intestine. Note the distension of the gut and the extreme thinness of the bowel wall at the site of perforation. At autopsy a peritoneal reaction was noticed, in the form of oedema and congestion of the mesentry, and free fluid in the peritoneal cavity. The foetus was a nonmacerated female, aborted at I9 weeks.

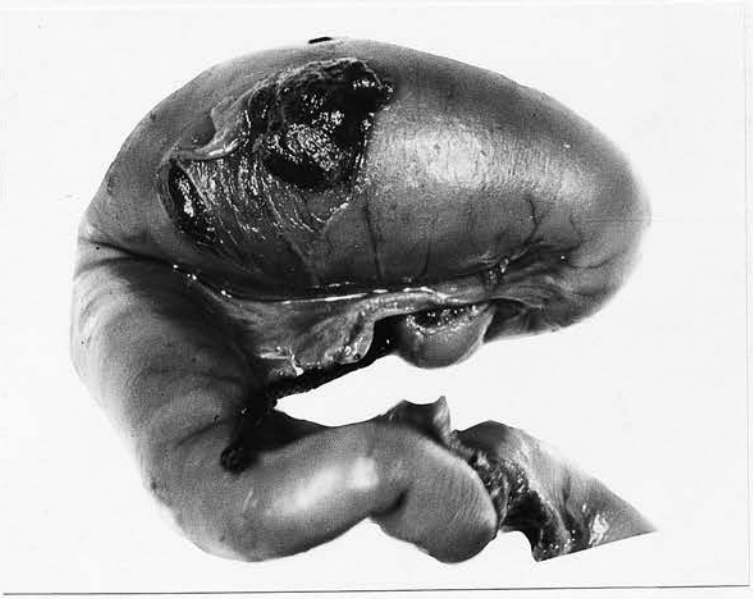

Fig. 2I

Fig. 22

A case of multiple congenital malformations (see Fig. I4), with the following cardio-vascular anomalies:-
persistent left superior vena cava - transposed aorta - pulmonary atresia - atresia of pulmonary artery - ventricular septal defect.

a

General cardio-vascular appearances. It is noted that the left innominate vein does not cross over to the right side.

b

The heart was pulled to the right to show the left innominate vein joining the persistent left superior vena cava. A probe was passed through, establishing continuity with the right atrium, which was enormously dilated and had an extension to the left behind the heart and the great vessels. The left atrium was very small, and received the pulmonary veins as usual.

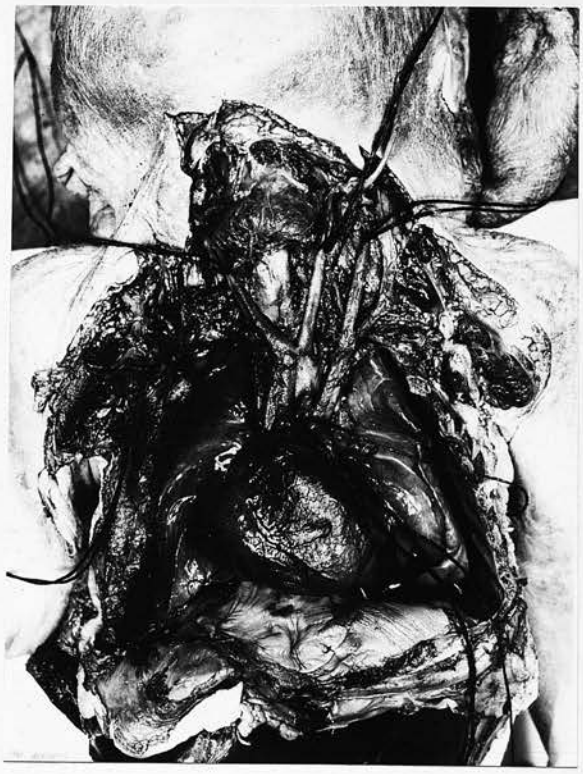

Fig. 22, a

Fig. 22, b

Fig. 22, cont.

c

The ductus arteriosus is seen, connecting the aorta and the (small) pulmonary artery, which externally looked as though arising by a common trunk. The pulmonary artery was slit open and a probe was passed along the lumen which was found to narrow down until it became completely atretic. Searching from the ventricular side, no pulmonary orifice could be found.

d

Diagrammatic representation of the findings in this case. The right atrium and the right and left superior vena cavae are shaded. Note the overriding aorta and the interventricular septal defect.

Fig. 22, c

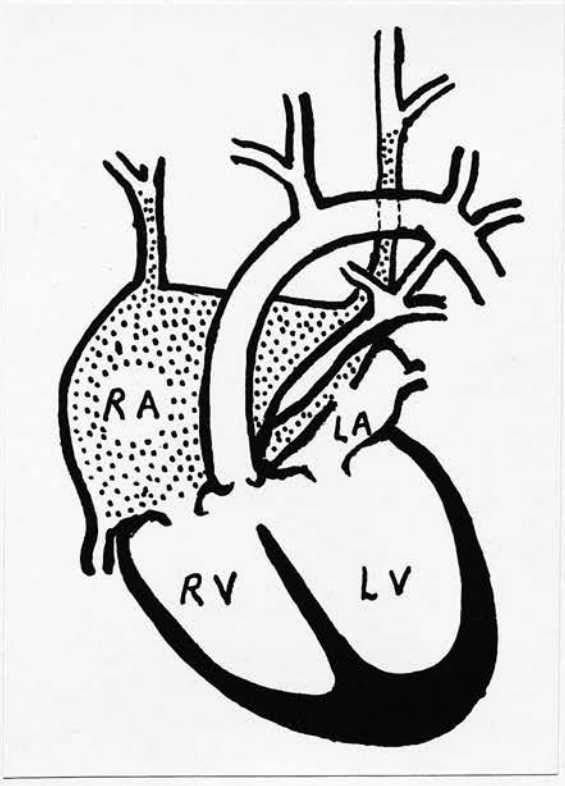

Fig. 22, d

Fig. 23, a

Arcuate demarkation of the ventricles. In the same case the ductus arteriosus was large, and looked as though it was the direct continuation of the pulmonary trunk which proceeded to join the aorta. The right and left pulmonary arteries stemmed out as side branches.

Fig. 23, b

Diagrammatic representation of the findings in the same case.
The foetus was spontaneously aborted at 24 weeks.
It also had a bilobed right lung and a club foot.

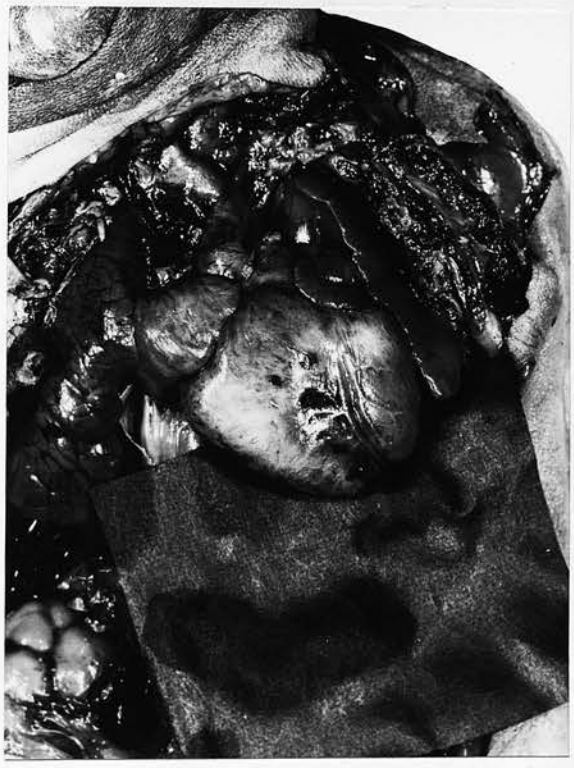

Fig. 23, a

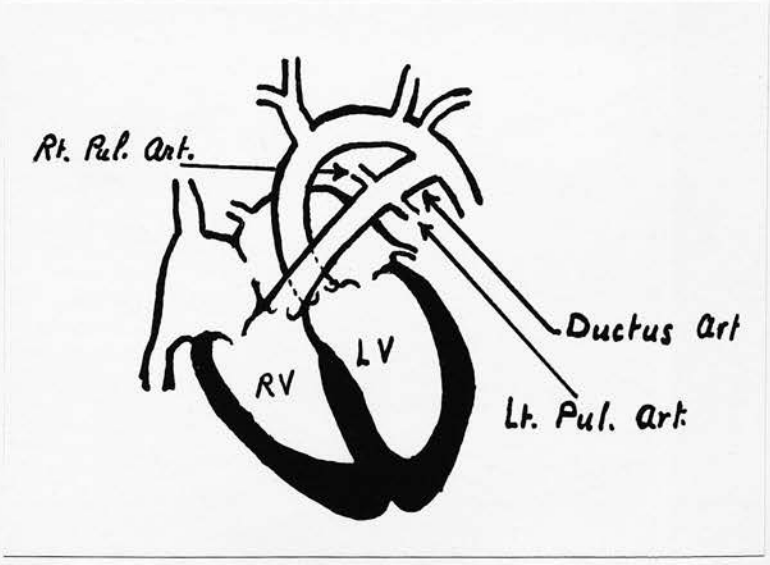

Fig. 23, b

Fig. 24

Bilateral hydroureter and hydropelvis. Note the dilatation and extreme tortuosity of the ureters and the dilatation of the renal pelves. The foetus, spontaneously aborted at 23 weeks, had also a talipes deformity of the left foot.
Patency of the urethra was established.

Fig. 25

Bilateral hydroureter and hydropelvis in one only (right) of identical twins aged 22 gestation weeks. Note the marked contrast with the normal urinary tract of the other twin.
The same foetus had a defect in the falx cerebri and persistent mesentry of the descending mesocolon.

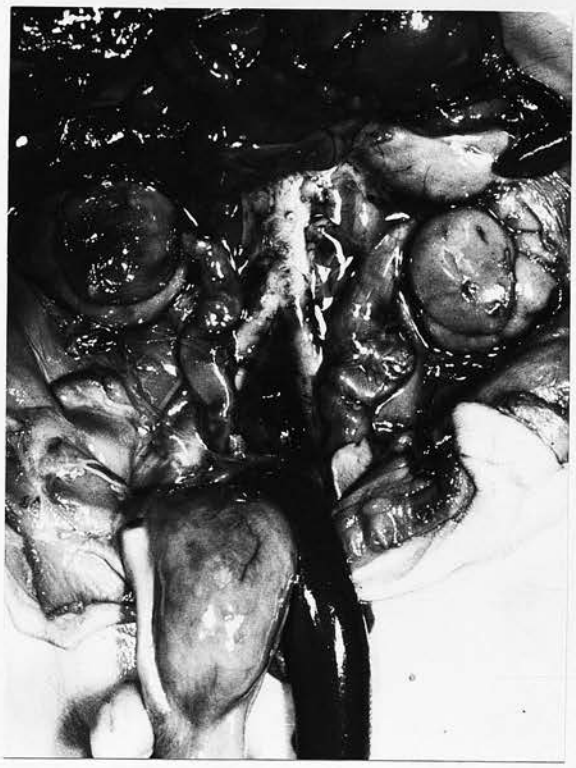

Fig. 24

Fig. 25

Fig. 26

Another stage of hydroureter and hydropelvis, in a 22 week foetus. Note the distended pelvis, the narrow pelviureteric junction and the dilated tortuous ureter. The urethra was proved patent.

Fig. 27

Dilated tortuous ureters without dilatation of the renal pelves. The urethra was patent. Spontaneous abortion at 24 weeks.

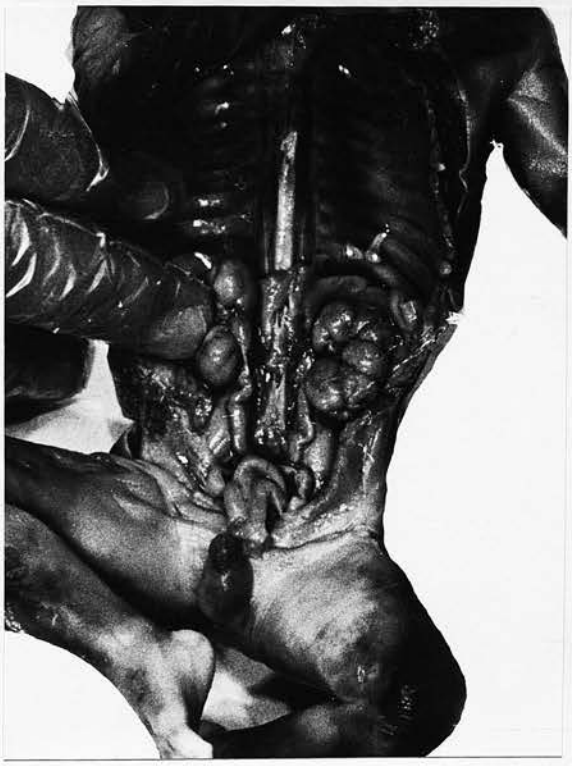

Fig. 26

Fig. 27

Fig. 28

Hypertrophy of the kidneys - microureters.
The histological picture of the kidneys was normal.
There were multiple congenital malformations (see Fig. I4).

Fig. 29

Cyst in the wall of the urinary bladder. On palpation from the outside the cyst felt so tense that it was thought to be a stone or a tumour.
Histological examination revealed a lining of transitional epithelium. Spontaneous abortion, 2I weeks.

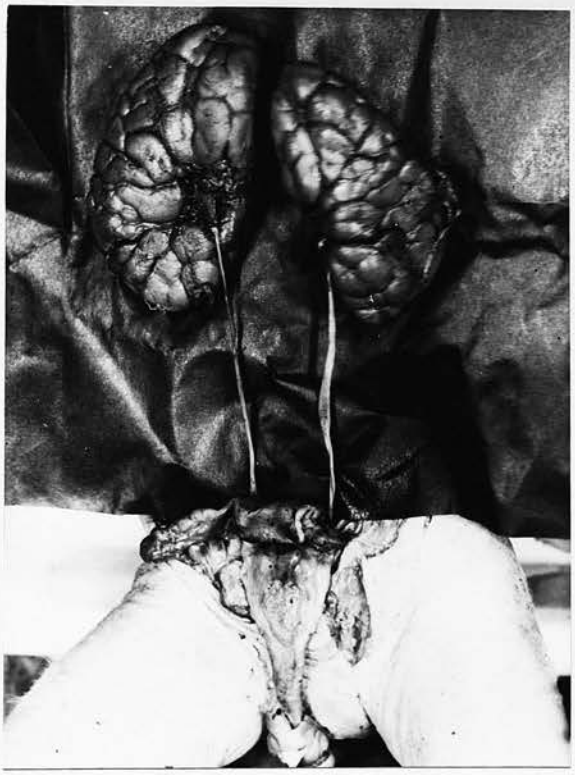

Fig. 28

Fig. 29

Fig. 30

Spina bifida. The gap in the lumbar and lower dorsal region was surrounded by a transparent gelatinous cutaneous bleb. There was also hydrocephalus (Fig. 34).

Fig. 31

Another example of spina bifida. Spontaneous abortion at 20 weeks.

Fig. 30

Fig. 3I

Fig. 32, a

Spina bifida occulta in a female foetus spontaneously aborted at I9 weeks. The covering skin was thin and deep blue in colour, and the defect was easily palpable.

Fig. 32, b

The same specimen after incision of the skin. The soft tissues were deficient, leaving a circular gap over the boney defect

Fig. 32, a

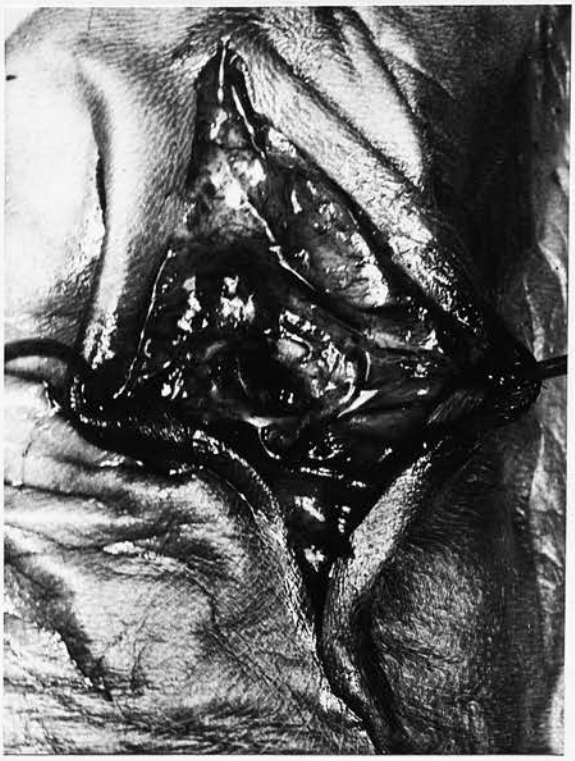

Fig. 32, b

Fig. 33

Encephalocele. Illigitimate pregnancy. Missed abortion, spontaneously terminating at 25 weeks. There was advanced maceration and the brain was completely liquified. The left lung was trilobed. Note also the short cord with atresia at the umbilical end. There was a single umbilical artery.

Fig. 34

Hydrocephalus. Same foetus as in Fig. 30. On external examination there was no notable enlargement of the head. On dissection the skull bones were found to be very thin (notice black tape showing through the extremely thin parietal bone). Part of the cerebral hemisphere was removed to show the marked dilatation of the lateral ventricle.

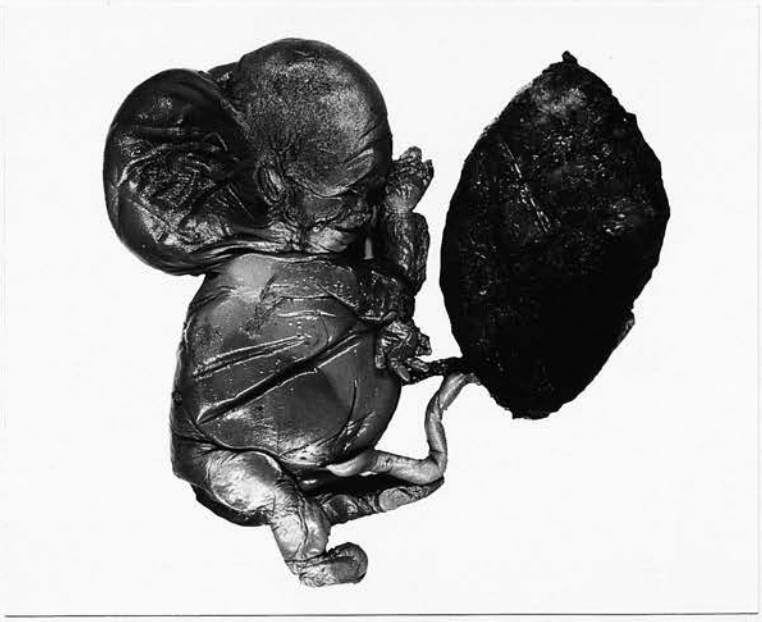

Fig. 33

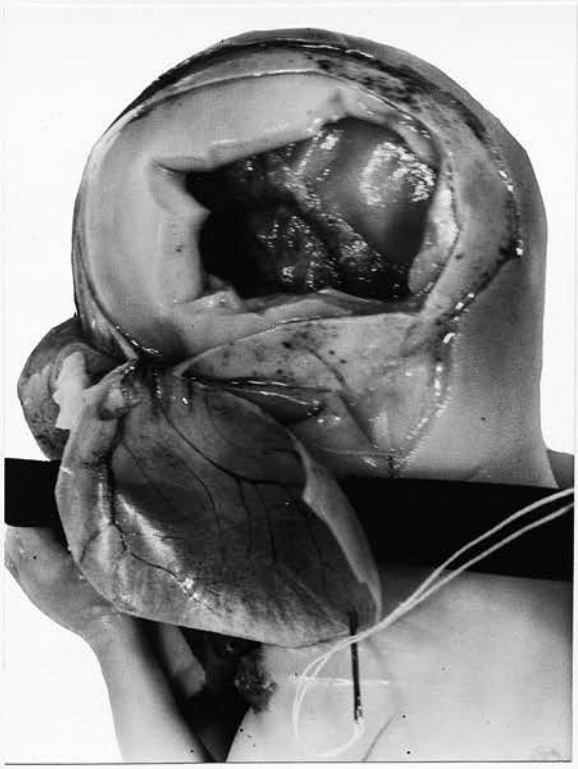

Fig. 34

Fig. 35

Fenestration of the falx cerebri in an II week foetus. The significance of this abnormality is not clear, but it has been described as part of I7-I8 trisomy syndrome.

Fig. 36

Another example of deficient falx cerebri. The margin of the defect contains a blood sinus. From a spontaneously aborted 22 week foetus, which also had hydroureters and hydropelves, and a persistent descending mesocolon.

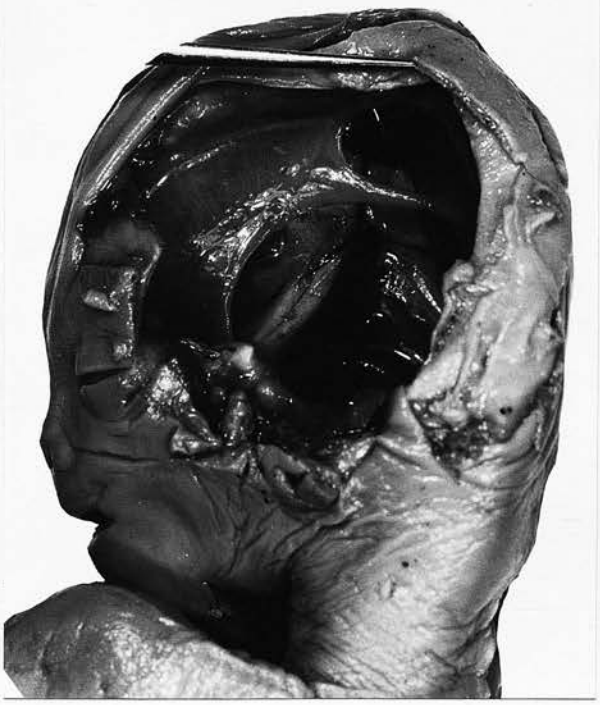

Fig. 35

Fig. 36

Fig. 37

Abnormal lobulation of the lung. The left lung consists of three lobes. I have been told by Dr. Barret of the Anatomy Department that such abnormalities of lung lobulation are a common dissecting-room finding. The practical implications are, however, obvious, especially in relation to thoracic surgery.

Fig. 38

Abnormal lobulation of the right lung, consisting of two lobes only. The left lung was normal.
Note also the volvulus of the small intestine.

Fig. 37

Fig. 38

Fig. 39

A nodule in the suprarenal gland in a 20 week foetus. The histological picture was that of suprarenal cortical tissue.

Fig. 40

Hare lip and cleft palate. Note also that the right hand bears six finger processes. The lower limbs are malformed and underdeveloped. Spontaneous abortion at 13 weeks.

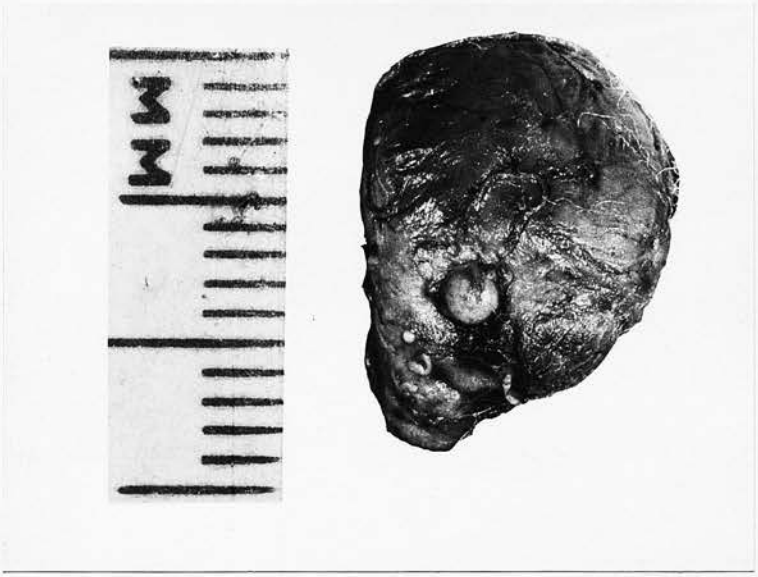

Fig. 39

Fig. 40

Fig. 41

Polydactyly. Same foetus as in Fig. 14.

Fig. 42

Micrognathus. Note the recession of the mandible. Spontaneous abortion at 14 weeks.

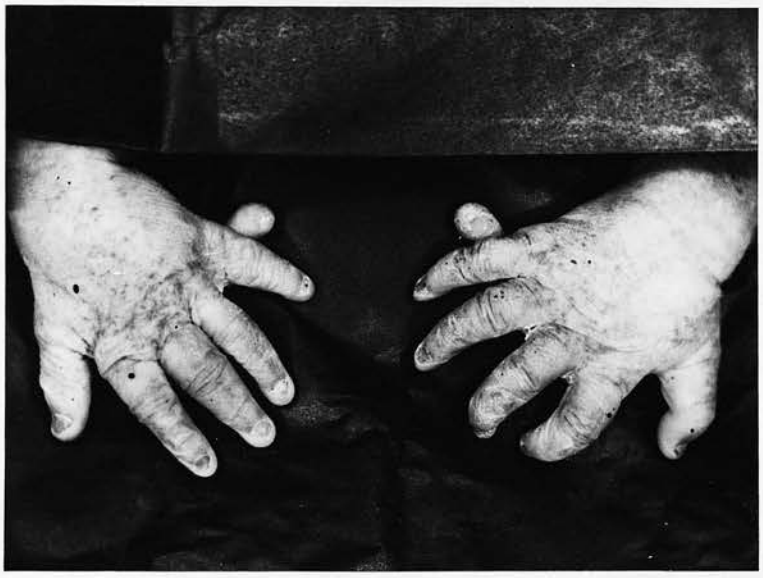

Fig. 4I

Fig. 42

Fig. 43, a & b

This foetus presents a number of malformations. There is a gap in the skull vault, leaving a membranous covering to the brain which is seen shining through. There is also marked recession of the mandible. The ears are low-set. The left upper limb shows phocomelia, and ends in one finger process.

Note also the torsion-stenosis of the umbilical cord at the abdominal end.

From a case of ectopic gestation treated by laparotomy at 8 weeks.

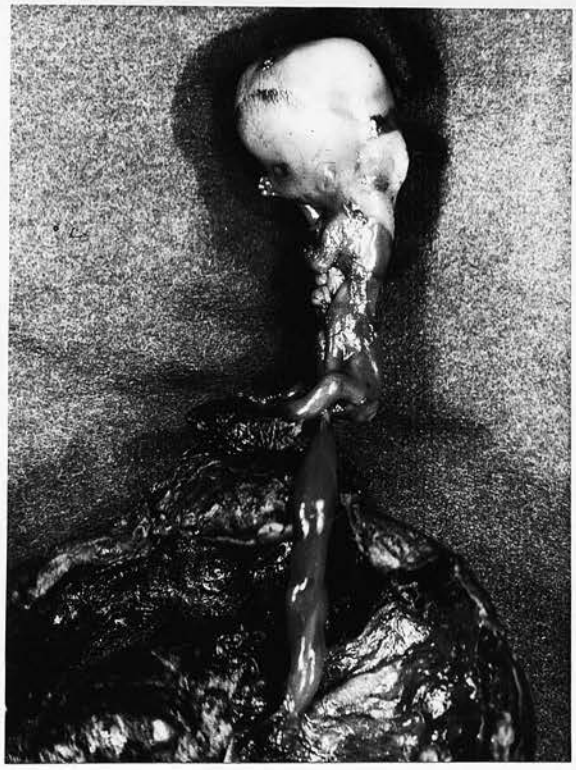

Fig. 43, a

Fig. 43, b

Fig. 44

Tail process in a foetus procured by laparotomy at I2 weeks from a case of ectopic pregnancy. Note also the vascular convolutions in the cord.

Fig. 45

Complete failure of development of the lower limbs (amelia). There is also failure of development of the fingers of the left hand as compared with those of the right. Spontaneous abortion at I3 weeks.

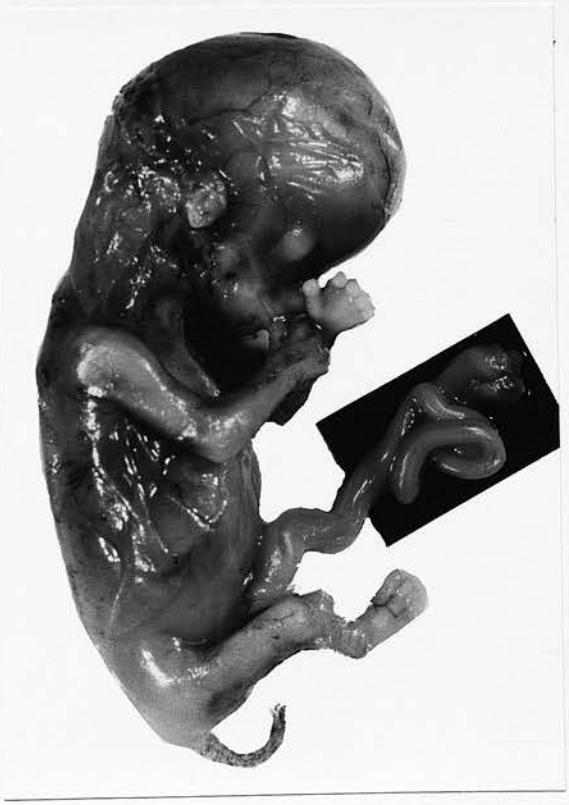

Fig. 44

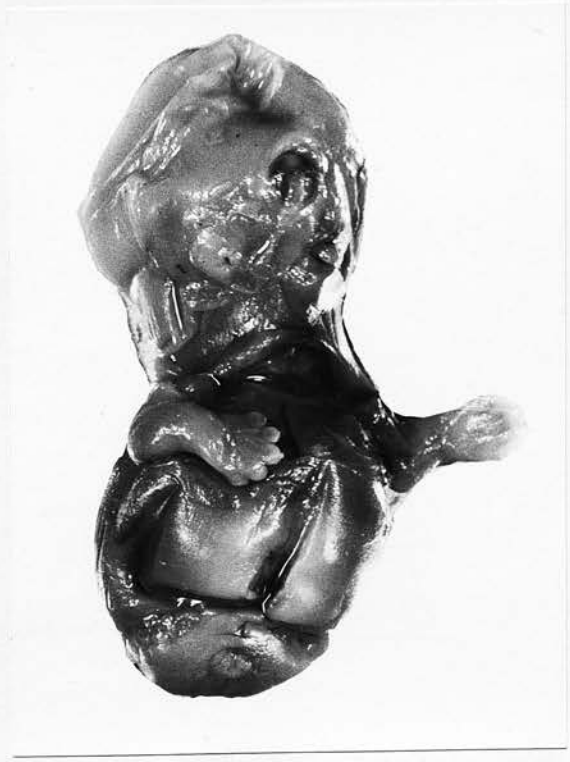

Fig. 45

Fig. 46

Asymmetrical growth of the lower limbs, the left being smaller than the right. The mother had multiple fibroids of the uterus, and was severely anaemic. Spontaneous abortion at I5 weeks.

Fig. 47

Multiple skeletal deformities of the upper and the lower limbs. The umbilical cord bears a cystic formation. Spontaneous abortion at I5 weeks.

Fig. 46

Fig. 47

Fig. 48

Advanced maceration with skeletal deformities of the left hand and foot.

Fig. 49

Multiple skeletal malformations. Torsion of the umbilical cord. From a case of missed abortion terminating spontaneously at 25 weeks. The mother was Rh negative with a high antibody titre. Only her first baby lived, after exchange transfusion. Since then, she had 3 Sb deliveries and a missed abortion before this pregnancy.

Fig. 48

Fig. 49

Fig. 50

The first two foetuses on the left show normal appearances. The third shows stunting of the lower limb buds, and the fourth is the foetus with absent lower limbs (Fig. 45).

Fig. 5I

Defective toes of the left foot. Case of missed abortion terminating spontaneously at I8 weeks. The foetus was markedly smaller relative to its menstrual age.

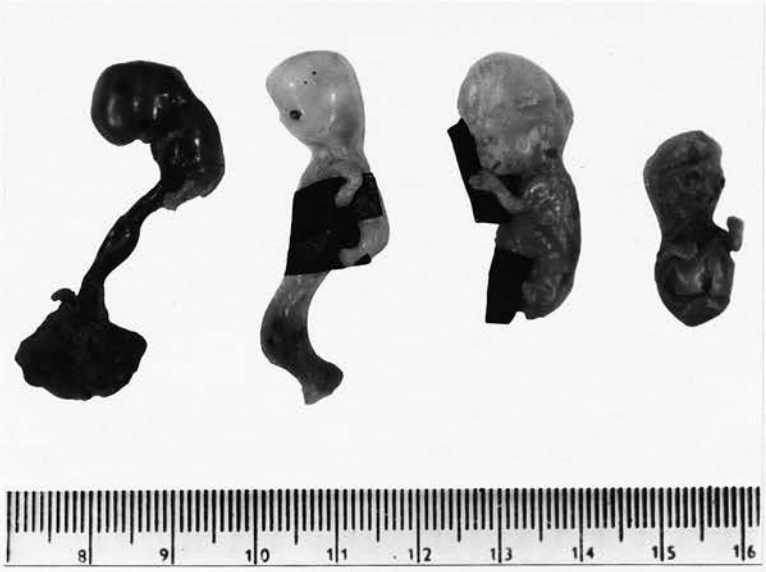

Fig. 50

Fig. 5I

Fig. 52

Hydrops foetalis.

Fig. 52

Fig. 53

Torsion of the umbilical cord. Missed abortion spontaneously terminated at 2I weeks.

Fig. 54

Torsion of the umbilical cord in association with umbilical hernia. From a case of missed abortion, induced with oxytocin drip at 2I weeks.

Fig. 53

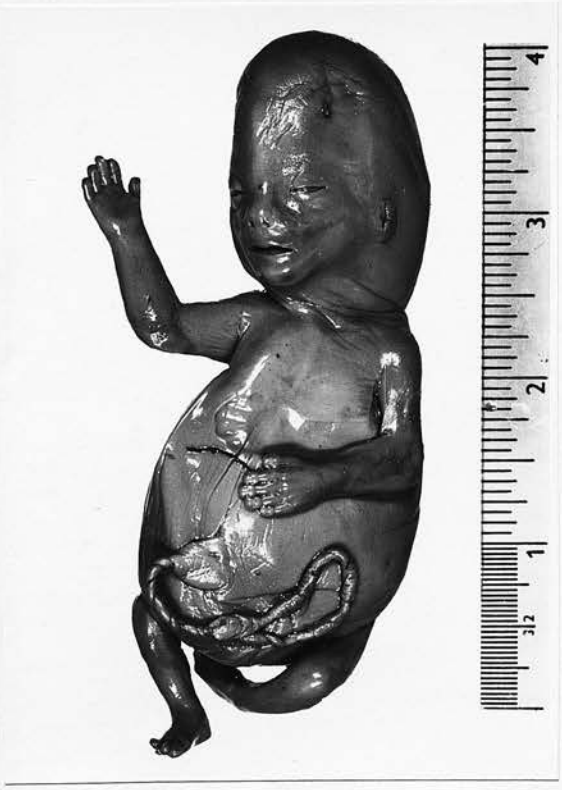

Fig. 54

Fig. 55

Constriction, cystic formation and torsion of the umbilical cord. Spontaneous abortion, I7 weeks.

Fig. 56

Constriction at the umbilical end and along the course of the cord. There is no abnormal torsion.

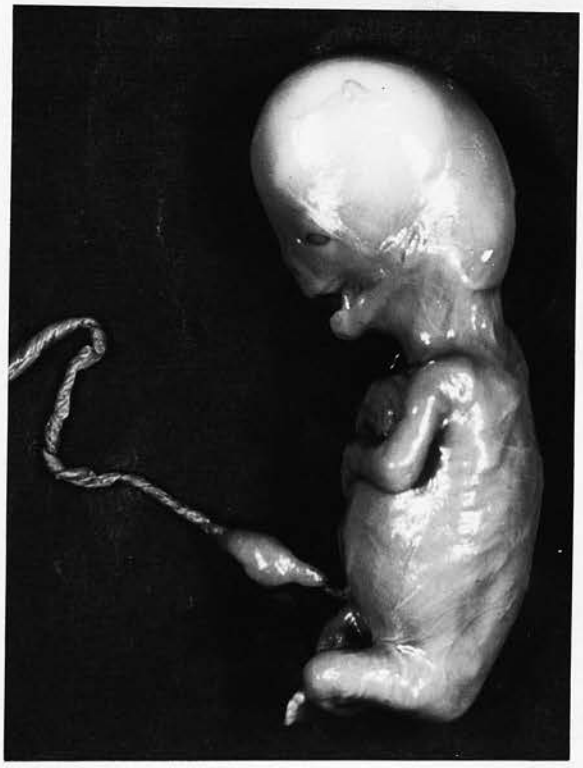

Fig. 55

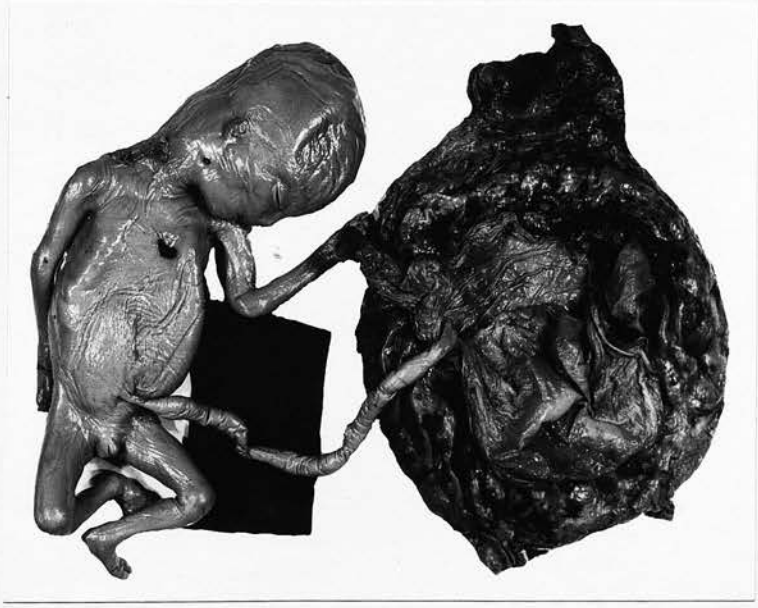

Fig. 56

Fig. 57

Abnormal looping of the cord around the neck of the foetus. The loops were quite tight. Strips of black paper have been inserted in between the loops.

Fig. 58

Abnormal looping of the cord around the neck. The specimen was received with no cord around the neck, but the cord has left a stigma in the form of the deep groove constricting the neck.

Fig. 57

Fig. 58

Fig. 59

Absence of the umbilical cord, leaving a nodule of friable tissue at the umbilicus. A possible cause in this case is torsion-amputation. The placenta was not available. Spontaneous abortion, II weeks.

Fig. 60

Cystic formation of the umbilical cord in association with a nodular embryo. The cord contained three vessels. Spontaneous abortion, II weeks.

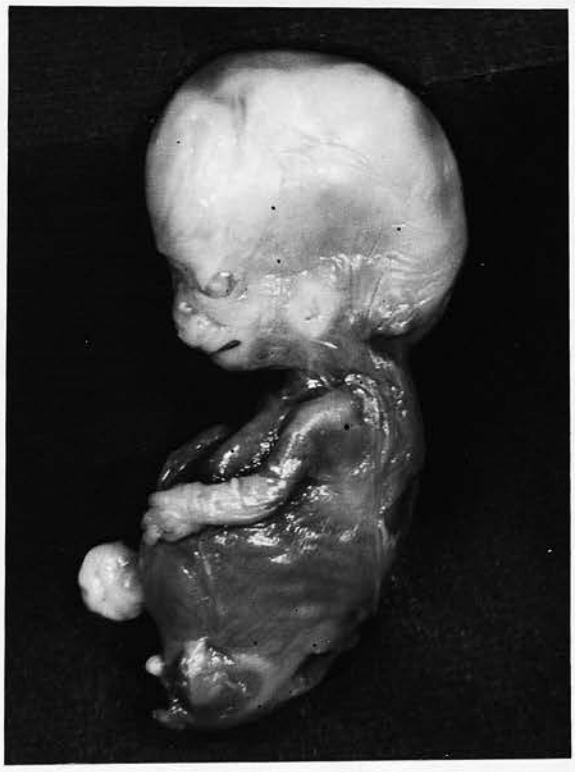

Fig. 59

Fig. 60

Fig. 6I

Placenta of uniovular twins, to show the mode of insertion of the cords. One cord has an excentric insertion (marked by a match stick) and the second a velamentous insertion. The placental share of the first is almost double that of the second. The heart of the first foetus weighed 9.I5 gm., that of the second second weighed 2.89 gm.

Fig. 62

'Mesentry' of the umbilical cord. Although the insertion of the cord was almost battledore, the fold of amnion gave the appearance of a nearly central insertion. This pitfall should be remembered when reporting cord insertion.

Fig. 6I

Fig. 62

Fig. 63, a

Nutrient vessels in the umbilical gord. It was the belief of some authors that the cord contained no vascular elements other than the umbilical vessels, and that it derived its own nutrition from the liquor. 'Vasa chordae' have been shown in a number of cords.

X I8

Fig. 63, b

The same vessels X 85.

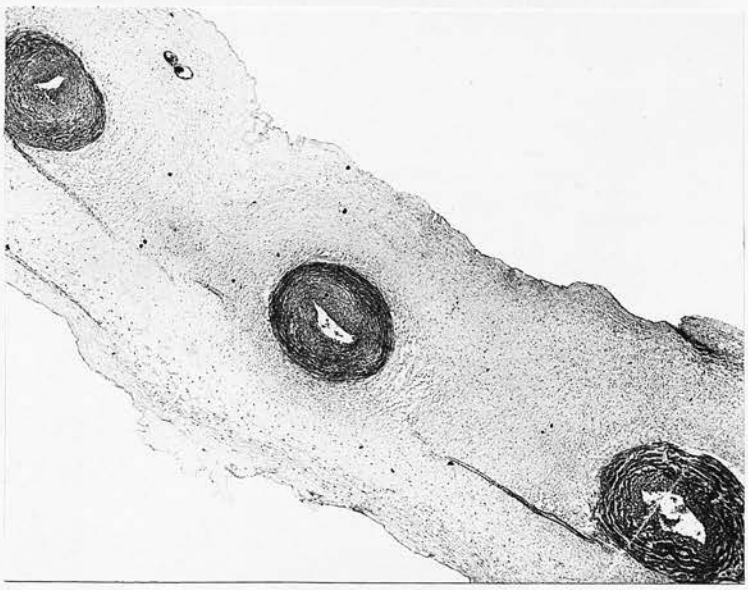

Fig. 63, a

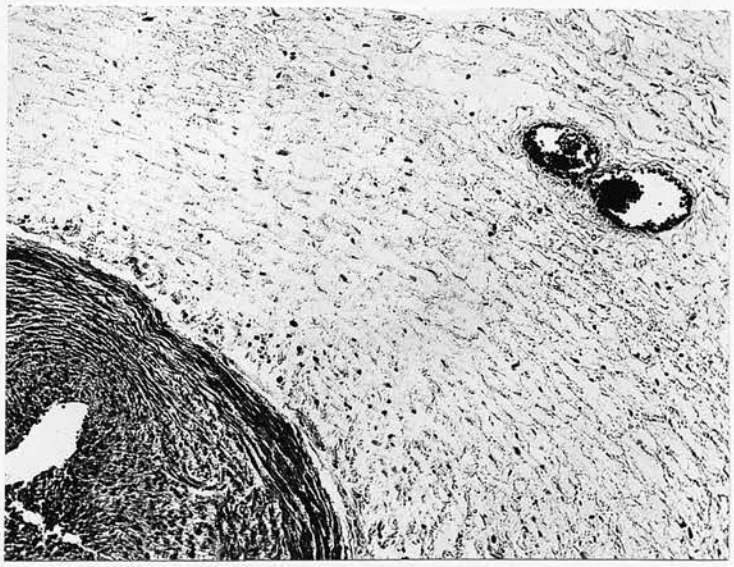

Fig. 63, b

Fig. 64

Absence of the umbilical vessels.

a

Small cord with a nodular embryo. Note the marked disparity in size between the gestation sac and that of the cord and embryo.

b

Section through the same cord, showing complete absence of the umbilical vessels. x 45

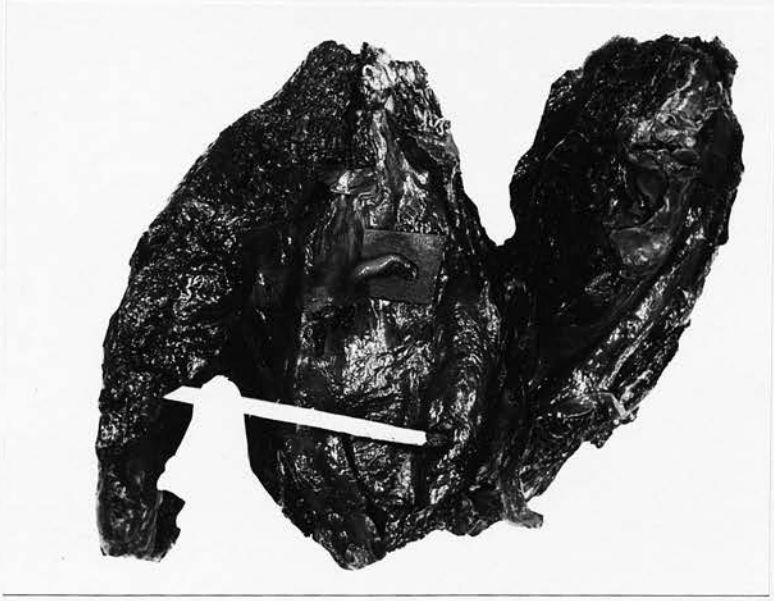

Fig. 64, a

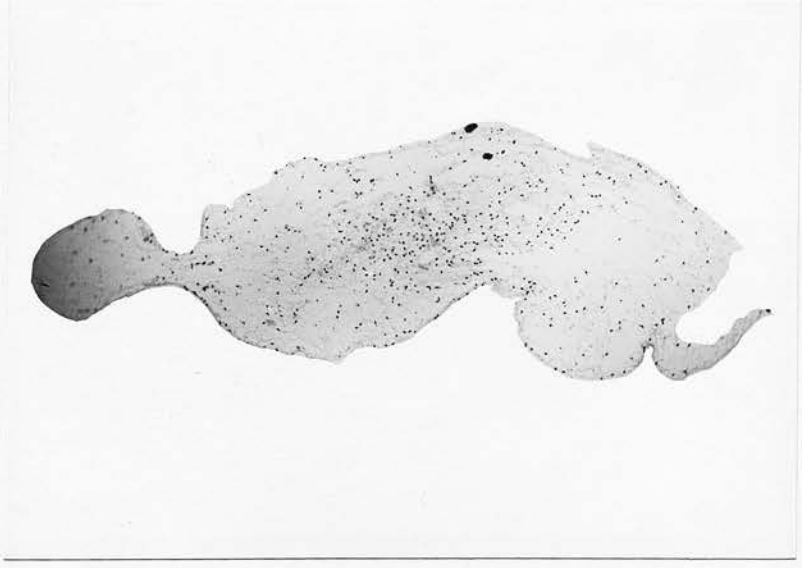

Fig. 64, b

Fig. 65

Single umbilical artery. There is also funitis, as shown by the leucocytic infiltration of the vessel walls and Wharton's jelly. x II0

Fig. 66

Missing umbilical artery - and nutrient capillary in the cord. There was also torsion of the cord.

x 25

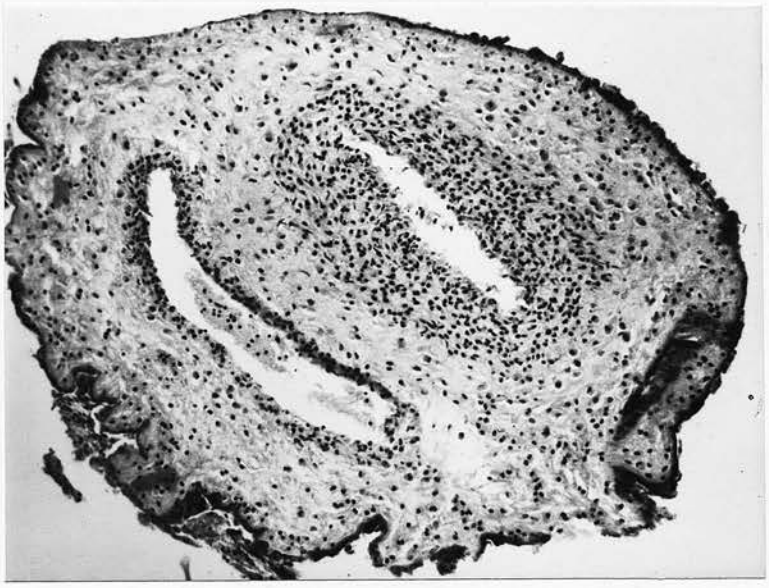

Fig. 65

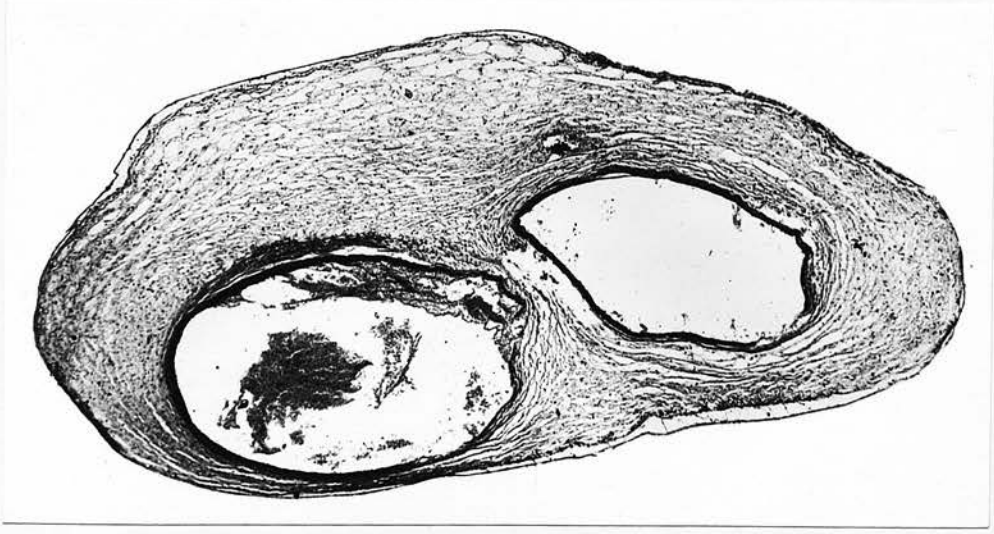

Fig. 66

Fig. 67

Anatomical dissection of a case of single umbilical artery. A length of black thread has been passed underneath the four iliac arteries after they were dissected. Both external and internal iliac arteries on the left side are much smaller in comparison with those on the right. The anterior branch of the left internal iliac artery was traced; it was found to become attenuated until reduced to a minute vessel fading alongside the urinary bladder, thus failing to proceed into the cord as an umbilical artery.

Fig. 68

Umbilical cord containing five vessels, probably due to branching of both arteries.

Fig. 67

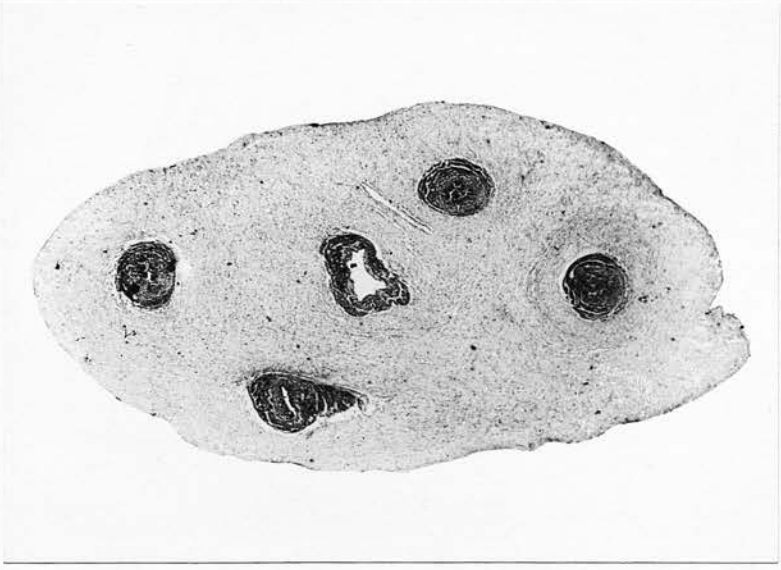

Fig. 68

Fig. 69

Inflammation of the umbilical cord "funitis". The Wharton's jelly and the vessel walls show marked leucocytic infiltration. x 60

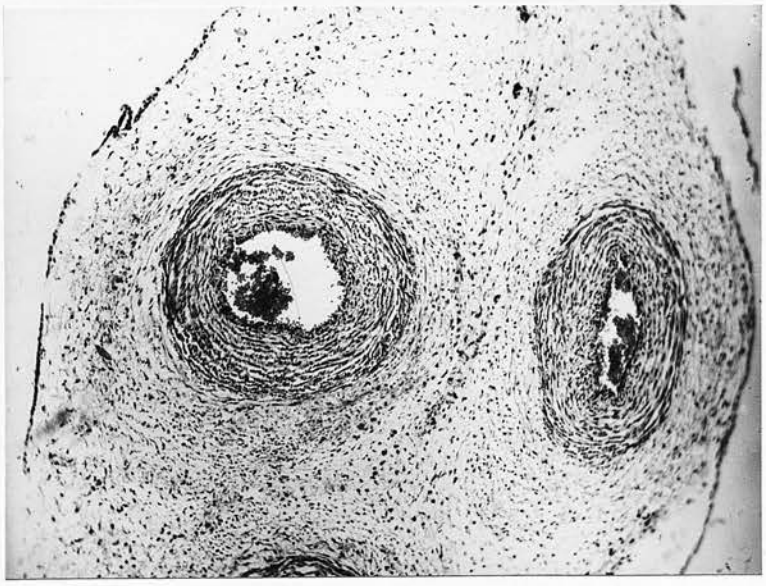

Fig. 69

Fig. 70

An abnormal placental form. An egg-shaped placental mass juts inside the amniotic sac, at the summit of which the cord is attached. Such a form must be a rare curiosity, and I could not find a report of a similar 'malformation'. Histologically it was formed of normal placental tissue.

a

Front view.

b

Profile view.

Fig. 70, a

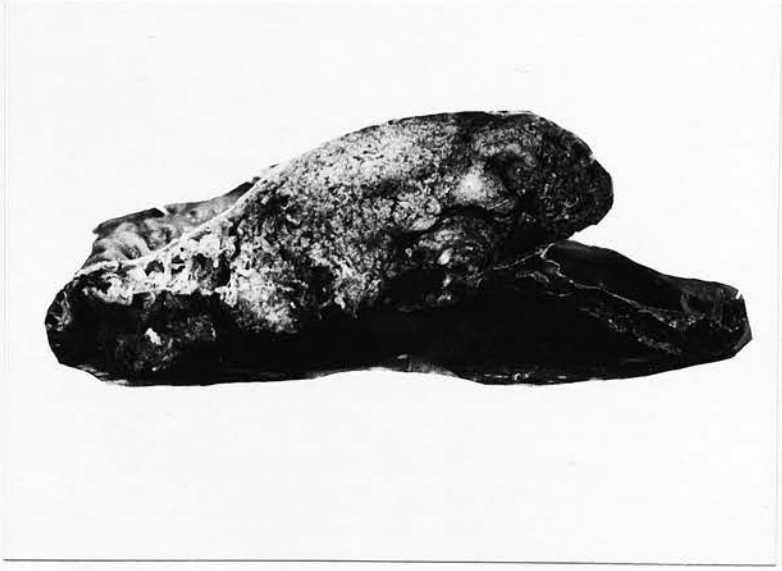

Fig. 70, b

Fig. 71

Placenta praevia. An intact gestation sac moulded to the shape of the uterine cavity, the bulk of the placenta occupying the lower pole.

Fig. 72

Placenta praevia. Diagnosed by the proximity of the site of rupture of the membranes to the placental margin. The gestation sac was reconstructed by packing with cotton wool, to show the relations as existed inside the uterus.

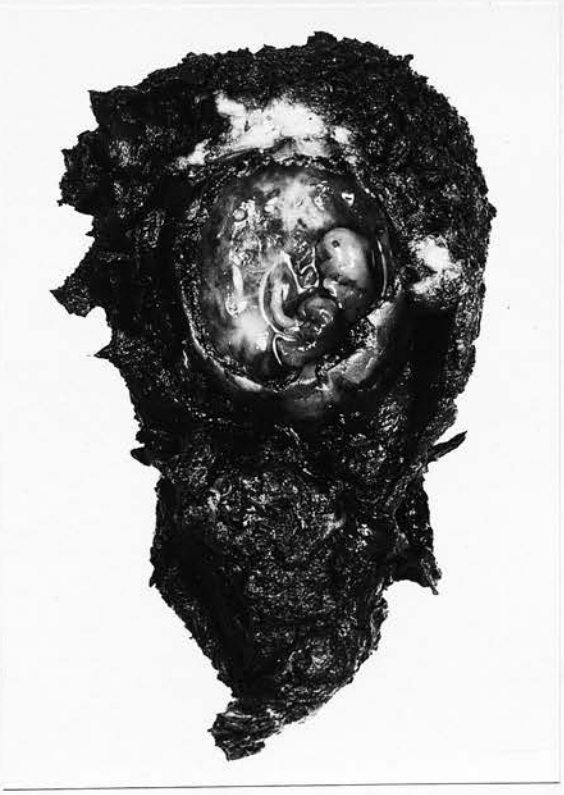

Fig. 71

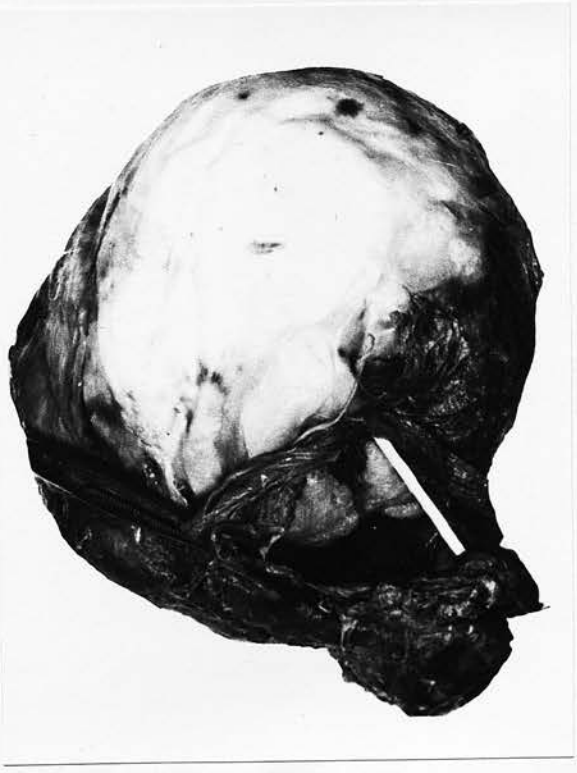

Fig. 72

Fig. 73

Placenta circumvallata.

Fig. 74

The site of a large retro-placental haematoma.

Fig. 73

Fig. 74

Fig. 75

Placenta marginata.

a
—

The inside of the sac.

b
—

The same sac from outside.

Fig. 75, a

Fig. 75, b

Fig. 76

Transitional mole. The vesicular change of the terminal villi was visible to the naked eye. There was a macerated embryo I2 mm. long. Microscopic examination showed hydatidiform degeneration.

Fig. 77

Pathological placenta. The maternal surface shows extensive white calcareous patches.

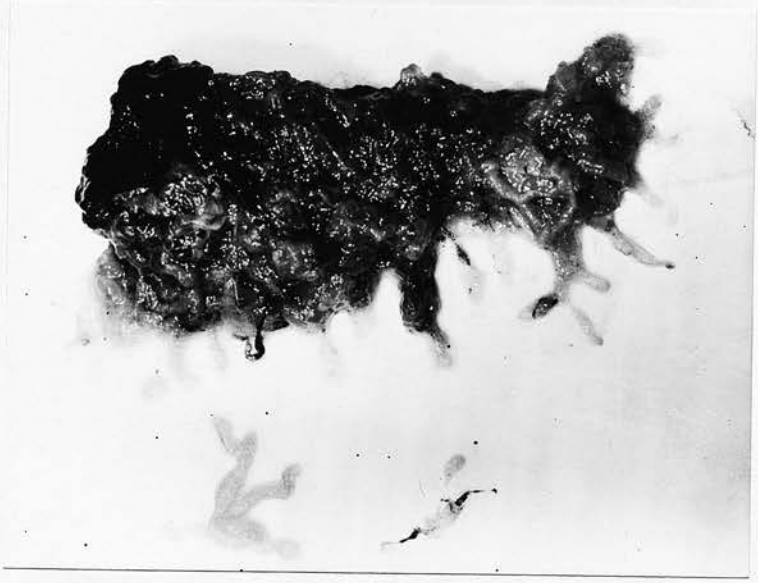

Fig. 76

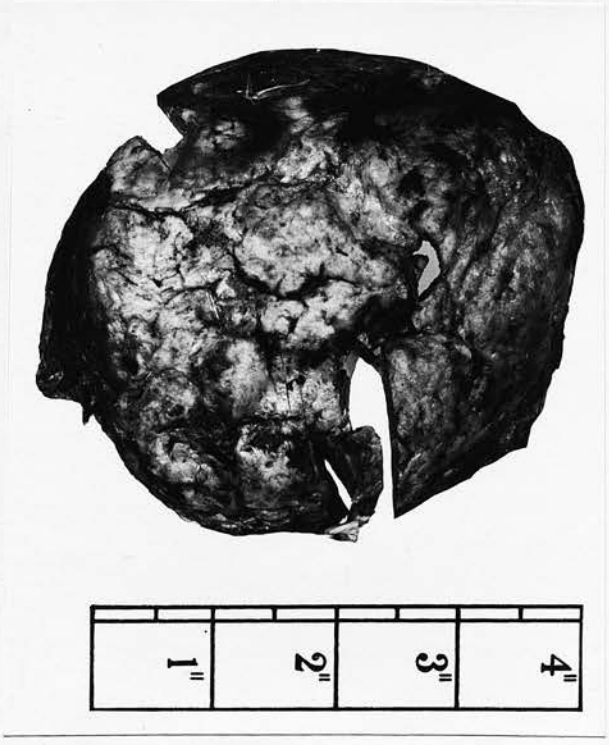

Fig. 77

Fig. 78

Macroscopic features of infection in the form of opacity and yellow discoloration of the membranes. The missing portion of membranes emphasises their opacity.

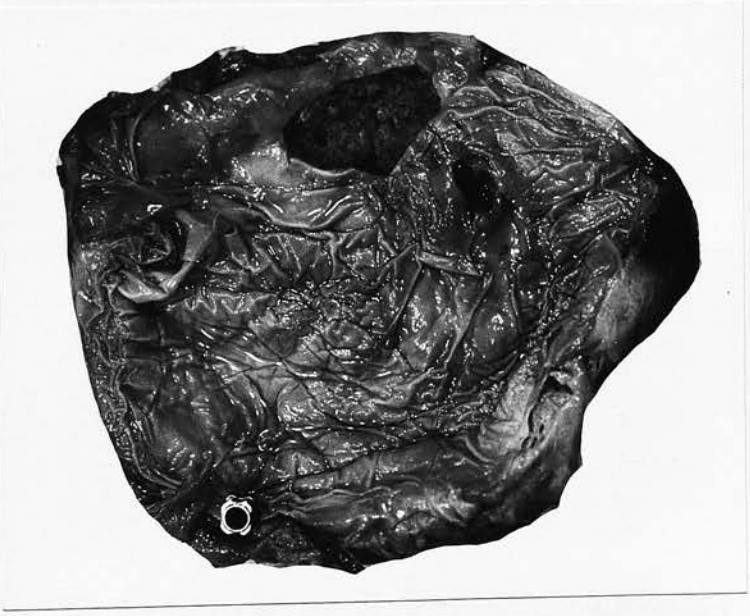

Fig. 78

Fig. 79

Normal early villus (7 weeks). Note the presence of both trophoblastic layers, and the location of the blood vessels inside the stroma, some distance from the trophoblastic covering. x I20

Fig. 80

Normal villus later on in pregnancy (I4 weeks). The cytotrophoblastic layer has largely disappeared leading to thinning of the trophoblastic covering. The blood vessels have approached the surface, lying close to the surface (margination). The intervening tissues between foetal and maternal circulations have become much thinner, which helps foeto-maternal exchange. x 95

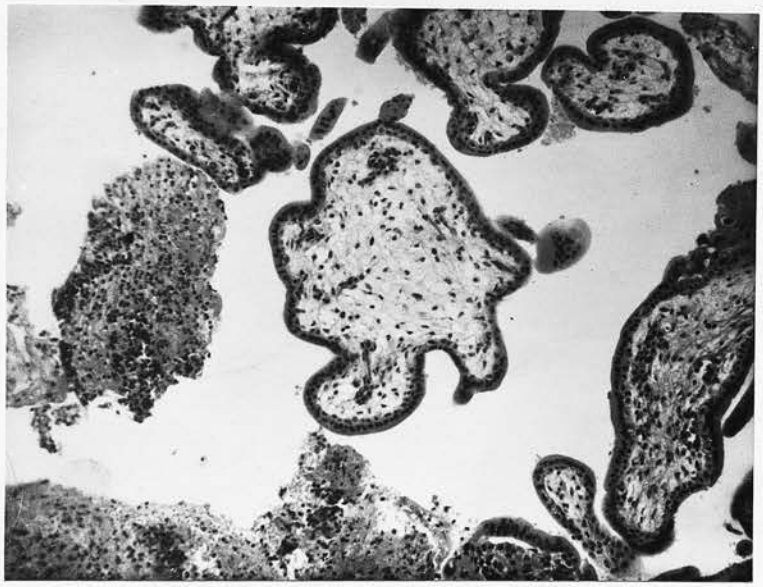

Fig. 79

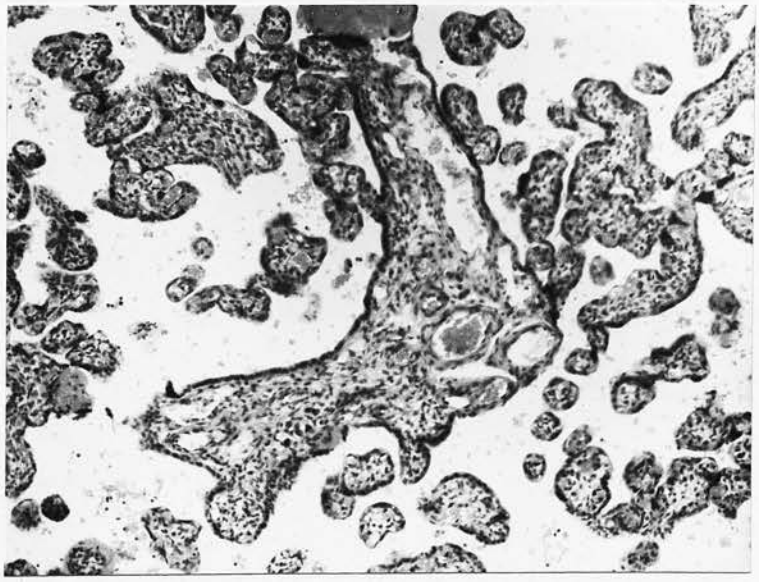

Fig. 80

Fig. 8I

Decidual haemorrhage. The bleeding can be traced to a maternal blood vessel in the decidua.
x I40

Fig. 82

Decidual haemorrhage. Note the formation of a laminated clot in the decidua. There is also leucocytic infiltration. x 90

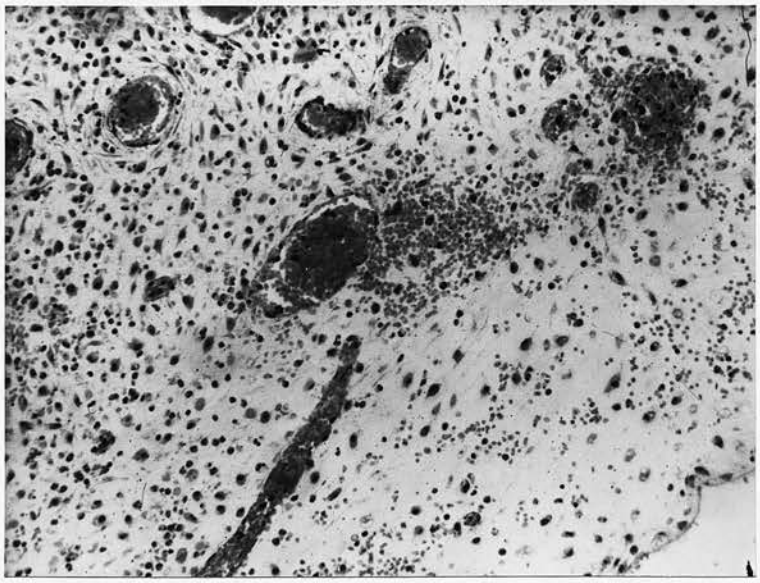

Fig. 81

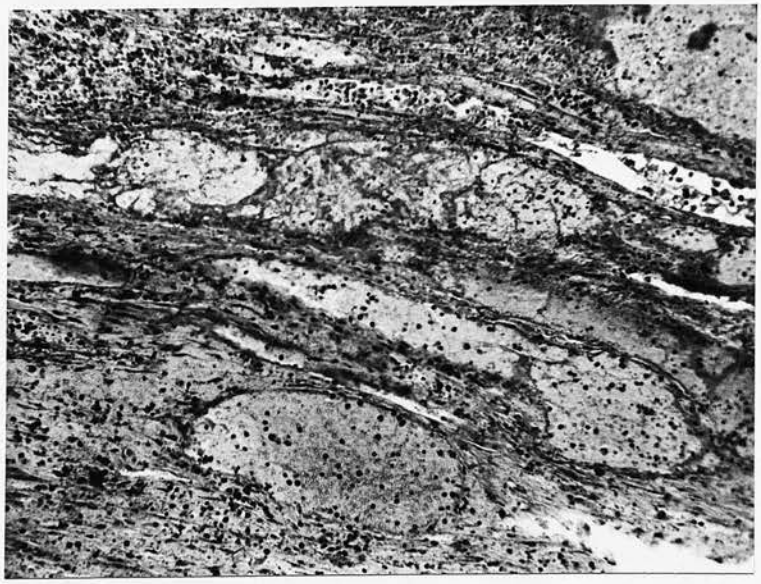

Fig. 82

Fig. 83

Haemosiderin deposits in an area of old decidual haemorrhage. x 90

Fig. 84

Intervillous haemorrhage. x 90

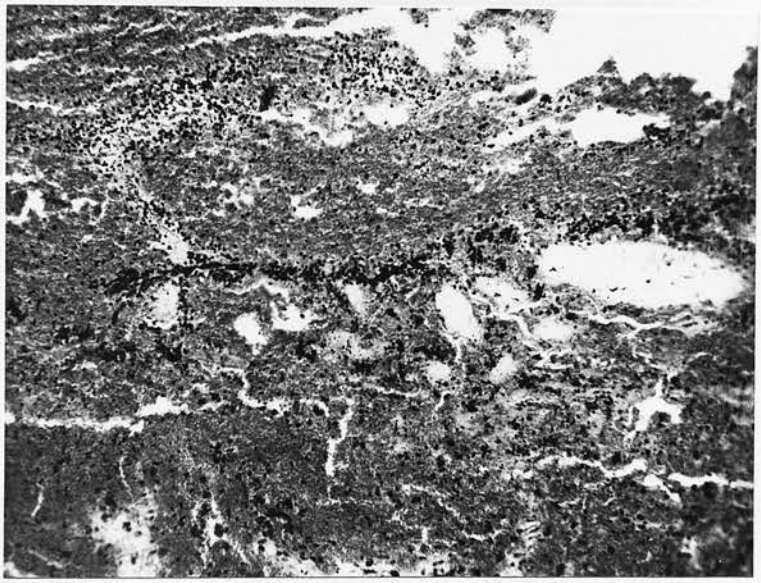

Fig. 83

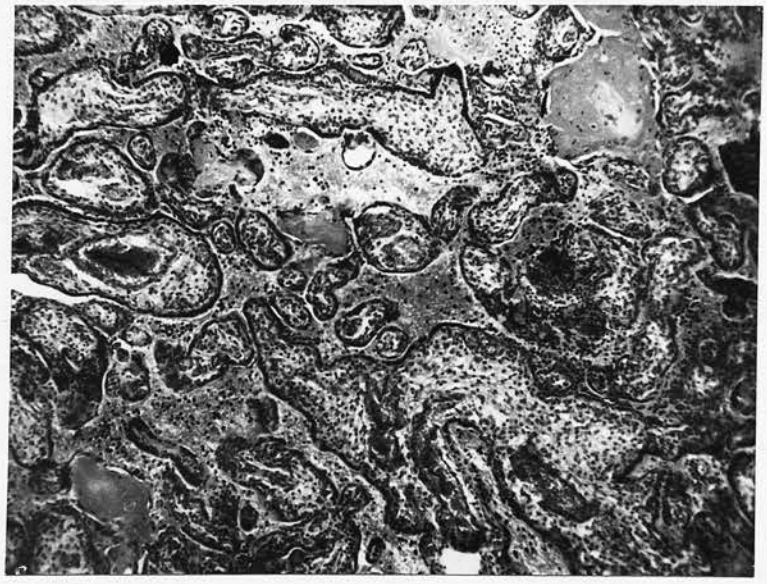

Fig. 84

Fig. 85

Breech of the villous wall leading to foetal bleeding into the intervillous space, with which the lumen of a foetal vessel is directly continuous. x I40

Fig. 86

Intravillous bleeding. Note the collection of blood in the lower half of the villous stroma, and the intact villous wall. x I20

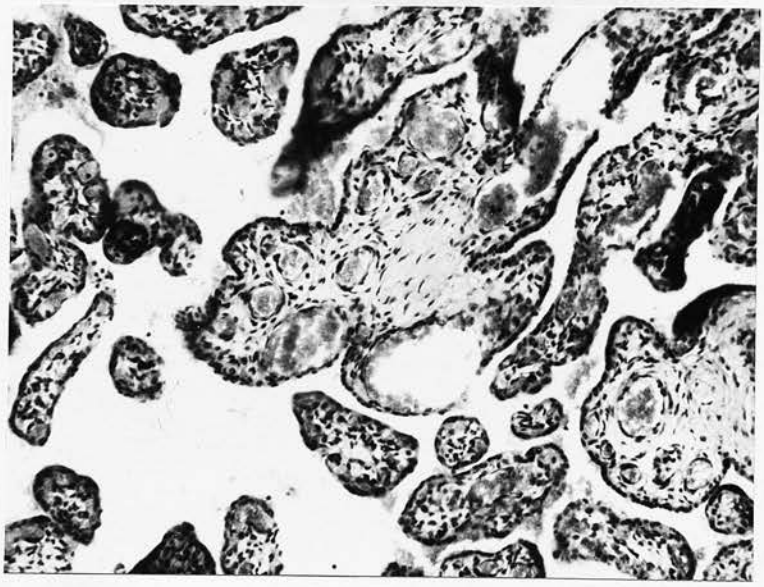

Fig. 85

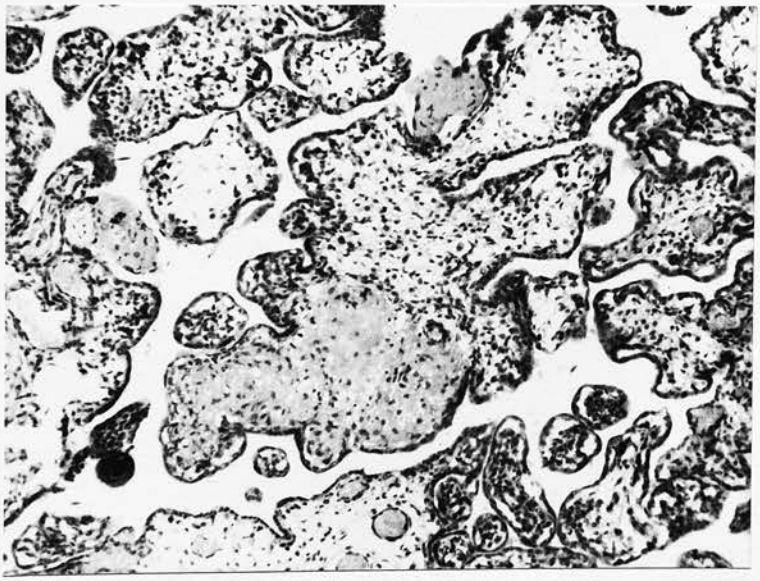

Fig. 86

Fig. 87

Fibrosing degeneration. The major part of the villous stroma is affected, leaving only a rim at the margin. x 105

Fig. 88

Simple villous oedema. The trophoblast is thin, and the (large) villus contains a blood vessel. x 80

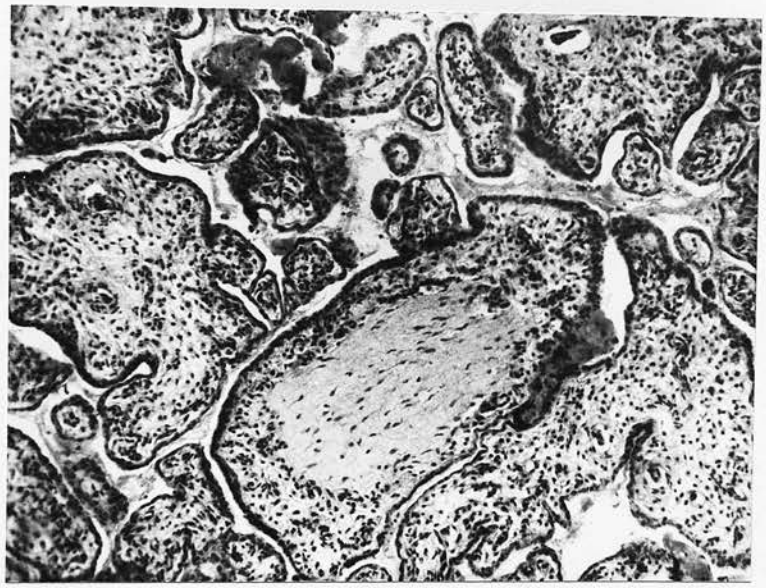

Fig. 87

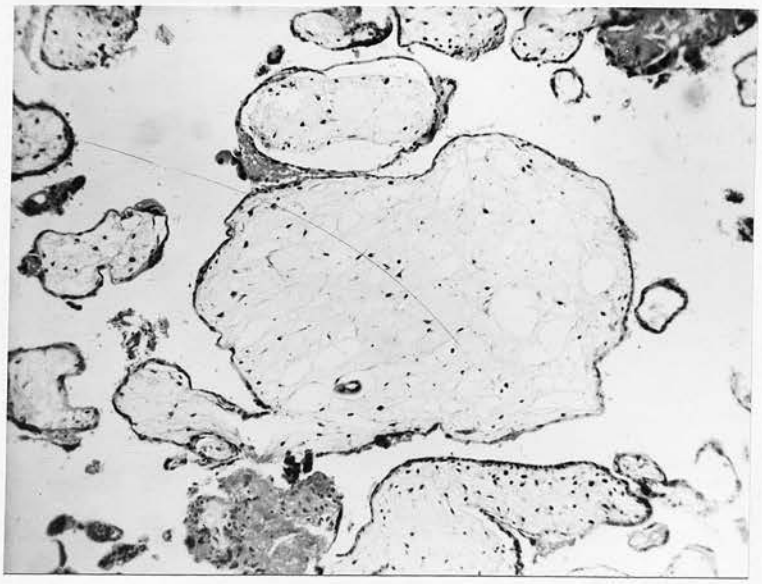

Fig. 88

Fig. 89

Hydropic degeneration. Note the marked recession of the stroma, and its complete disappearance in one villus. The villi are avascular. The trophoblast is not hyperplastic. x 105

Fig. 90

Transition stage between hydropic and hydatidiform degenerations. Note the trophoblastic hyperactivity. x 110

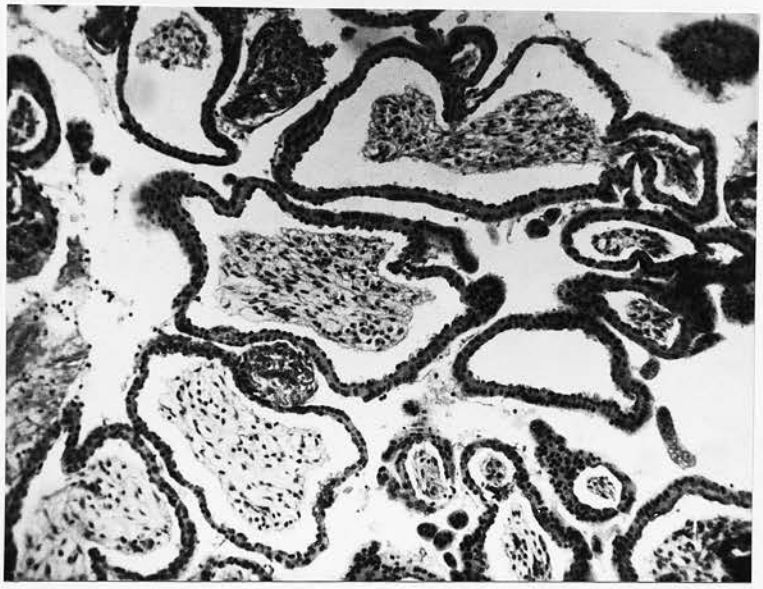

Fig. 89

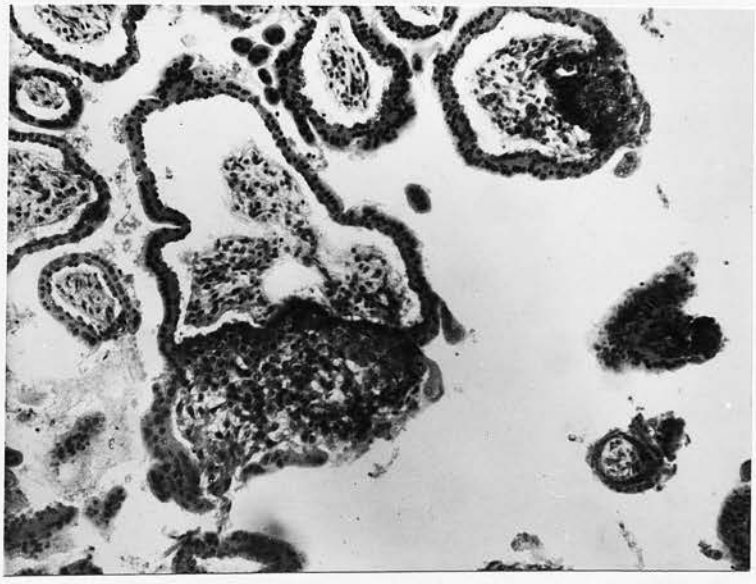

Fig. 90

Fig. 9I

Hydatidiform degeneration. x 60

Fig. 92

An early fibrinous mesh depositing around the villi.
x I00

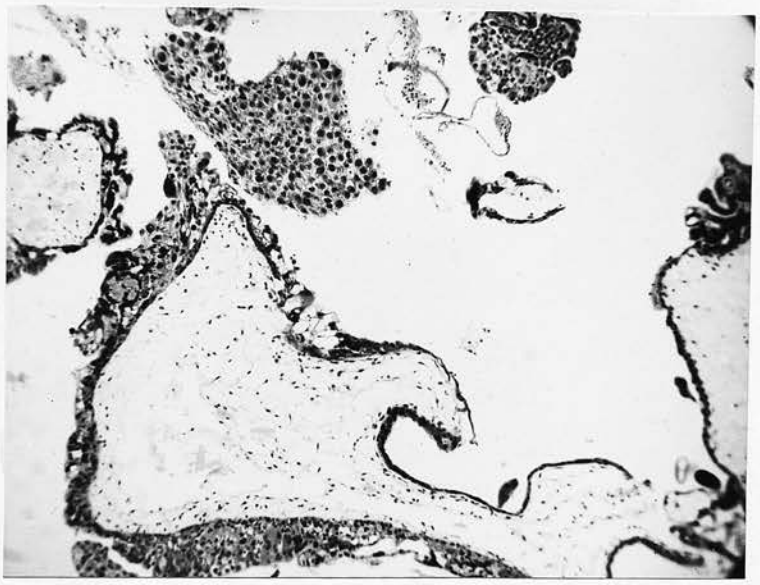

Fig. 91

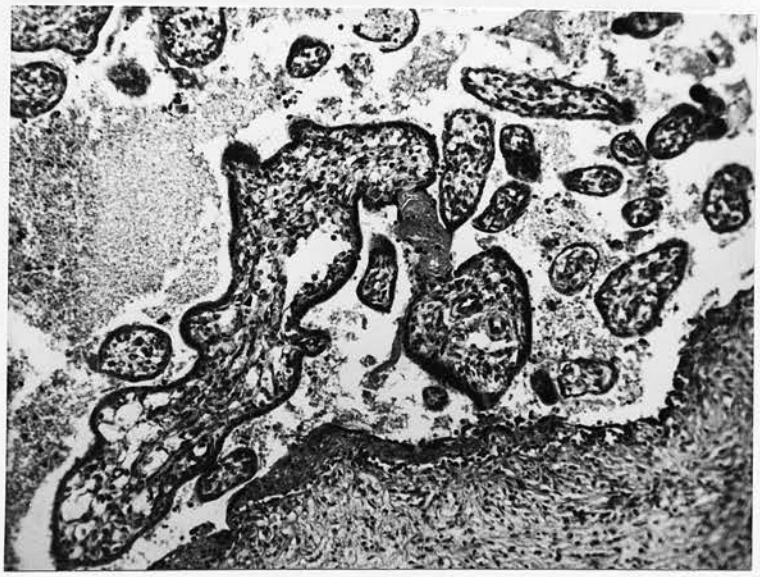

Fig. 92

Fig. 93

'Placental hypertrophy'. The villi are full of blood vessels, hardly leaving any space for the villous stroma. From a case of spontaneous abortion at 24 weeks; the foetus had multiple congenital anomalies. x 80

Fig. 94

Syncytiotrophoblastic islands free in the intervillous space. x 210

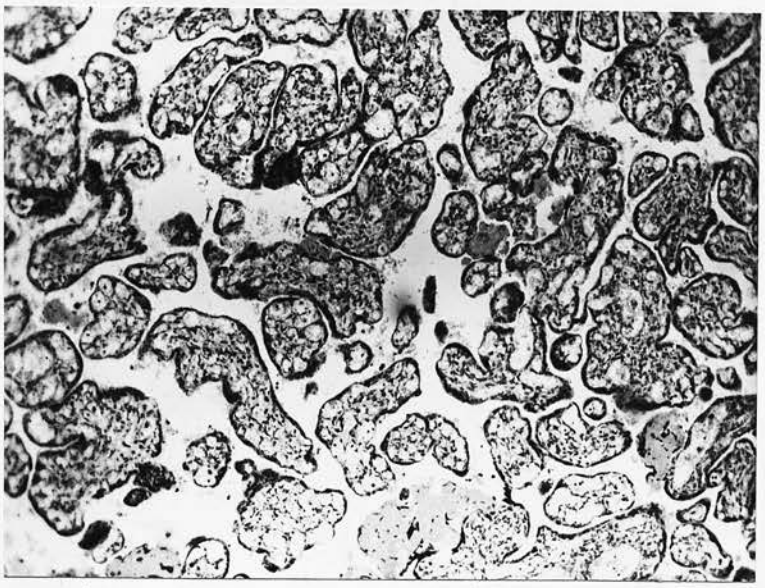

Fig. 93

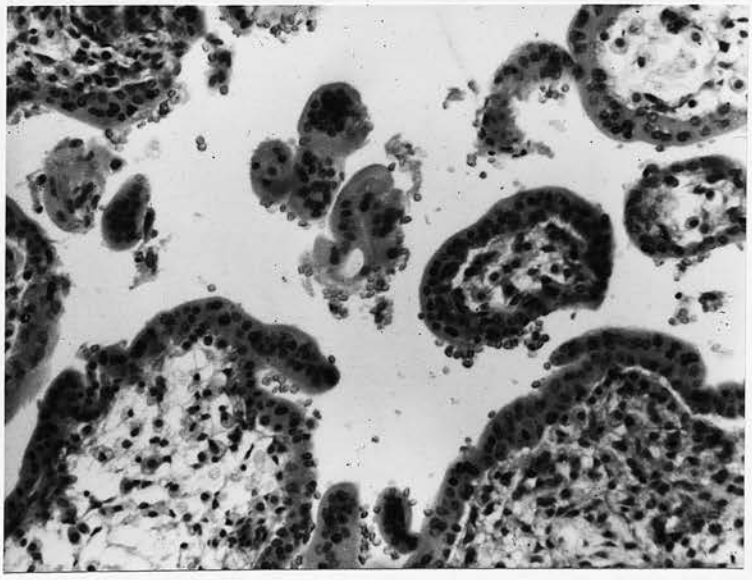

Fig. 94

Fig. 95

Trophoblastic islands in the form of giant cells in the decidua. x 210

Fig. 96

Trophoblastic 'columns' amongst the decidual cells. x 210

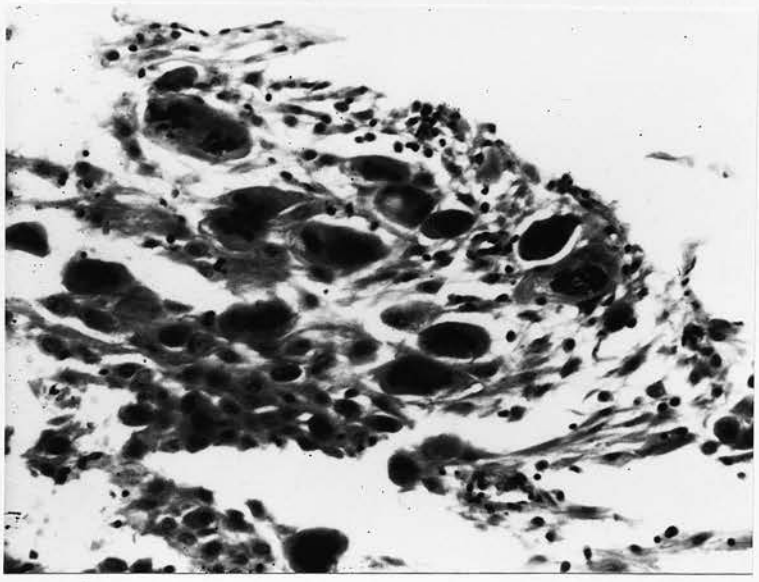

Fig. 95

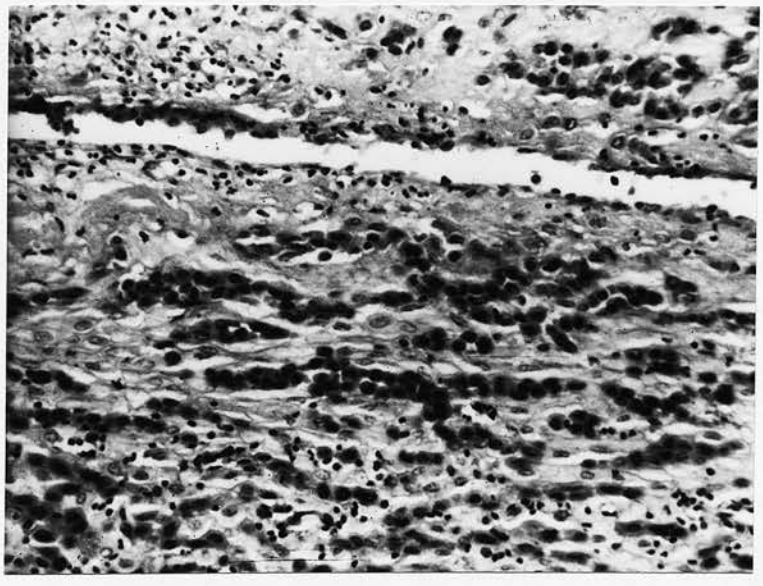

Fig. 96

Fig. 97

Infarction. The black areas denote calcification in degenerated trophoblastic knots. x80

Fig. 98

An advanced stage of infarction. There are only ghost villi amongst the fibrinous deposit. x 90

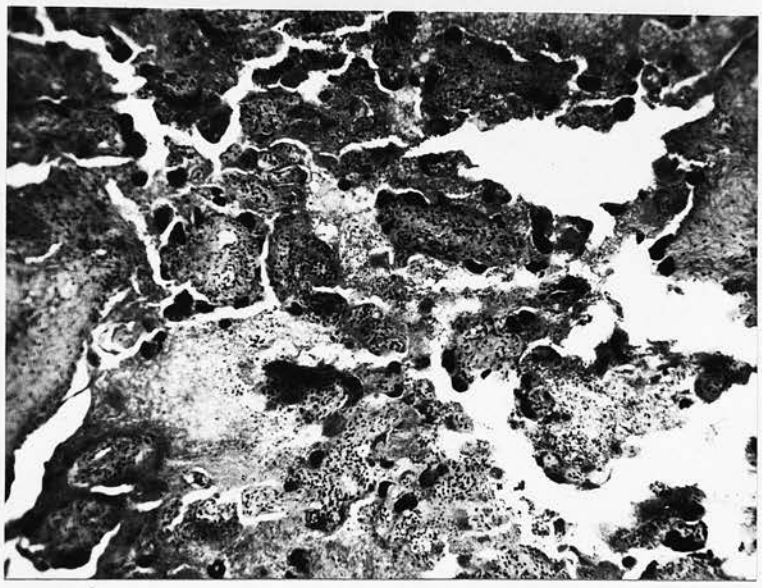

Fig. 97

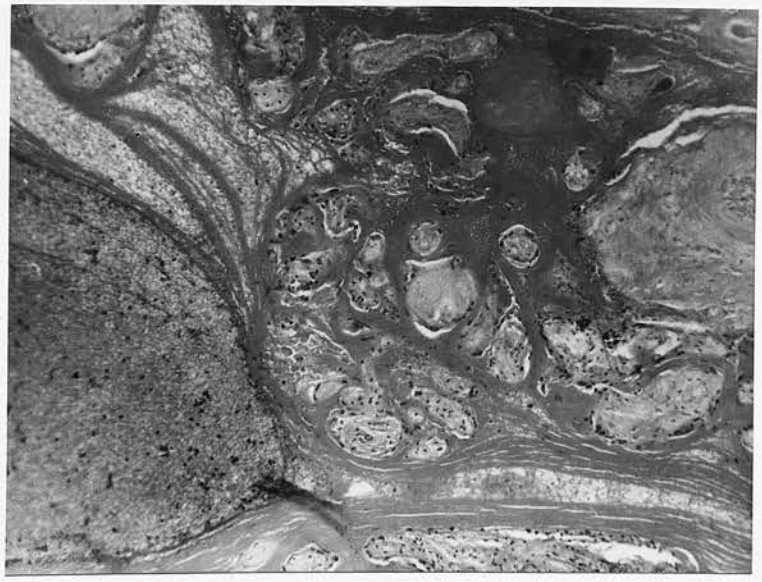

Fig. 98

Fig. 99

Infection. Note the marked leucocytic infiltration of the decidua. x I40

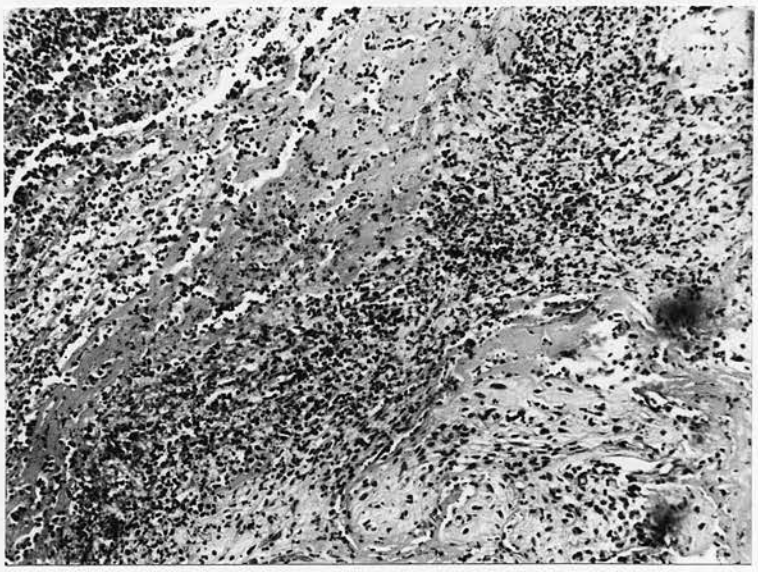

Fig. 99

Fig. I00

Normal membranes. x 80

Fig. I0I

Infection of the membranes. Note the marked leucocytic infiltration, in contrast with Fig. I00. x I25

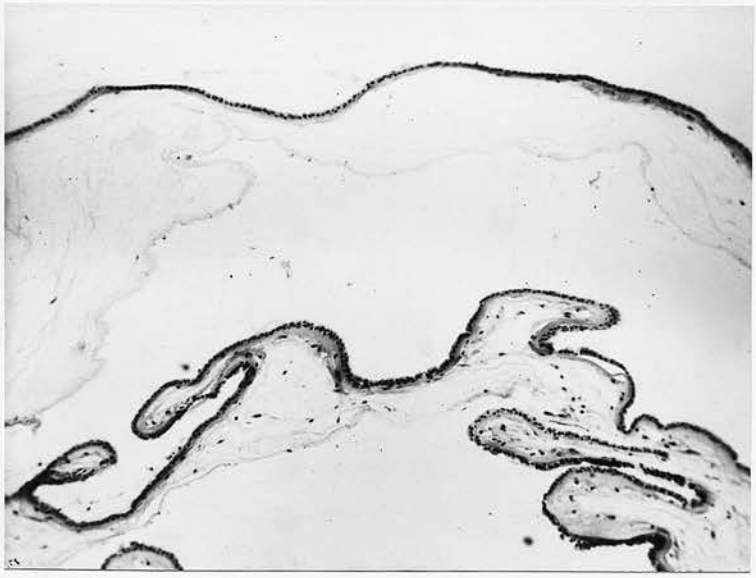

Fig. 100

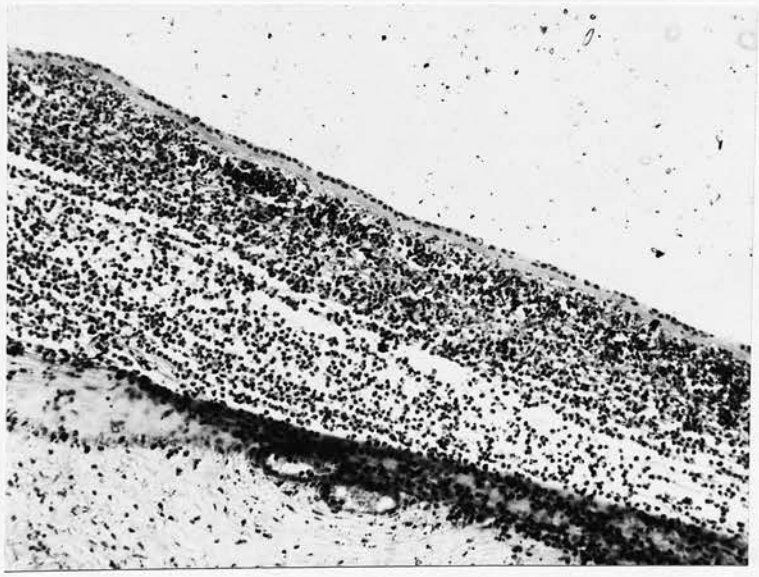

Fig. 101

Fig. I02

Barr body in the nucleus of a villous stroma cell.
x I500

Fig. I03

Barr body in a trophoblastic nucleus. x I500

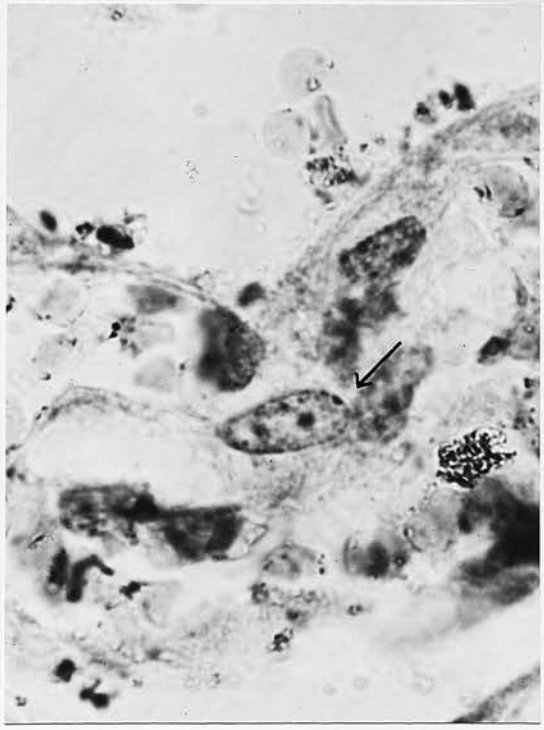

Fig. 102

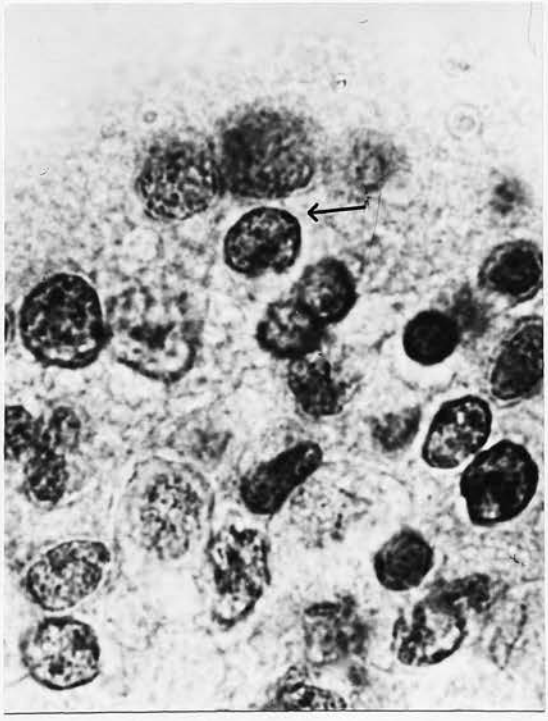

Fig. 103

Fig. I04

Barr body in the vascular endothelium lining a foetal vessel in a chorionic villus. x I500

Fig. I05

Barr body in a foetal erythroblast in a villous vessel. x I500

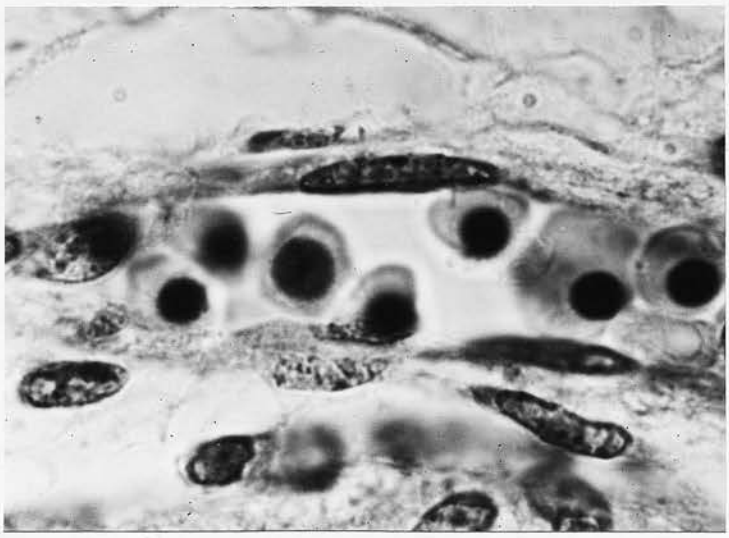

Fig. 104

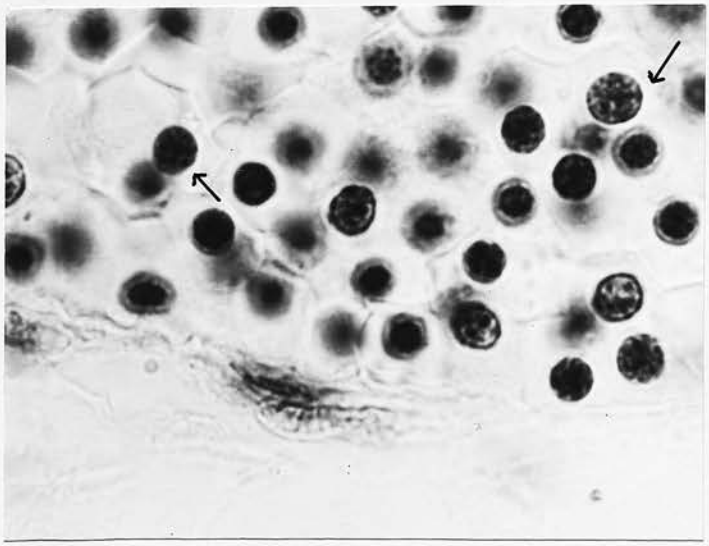

Fig. 105

Fig. I06

An amniotic fluid preparation showing Barr body in a squamous cell of a female foetus. x I500

Fig. I07

Double Barr bodies in a proportion of cells (7 per cent) of a female foetus with a high (68 per cent) Barr count. This denotes a sex-chromosome complement of XXX in at least some of the cells.
x I500

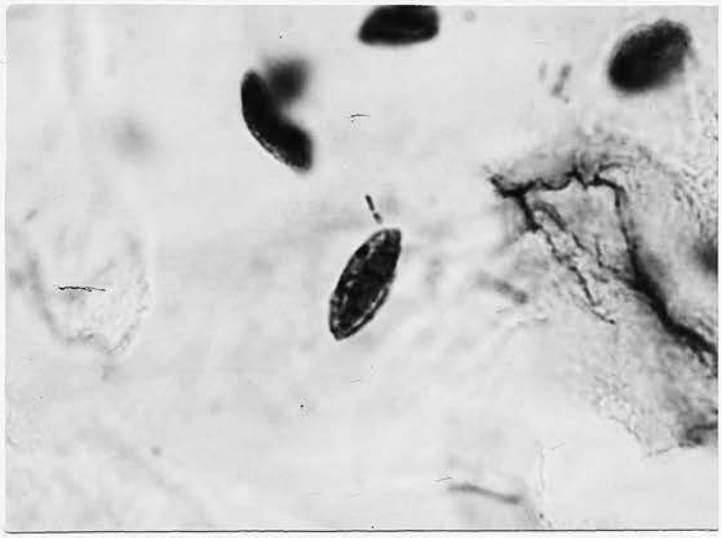

Fig. 106

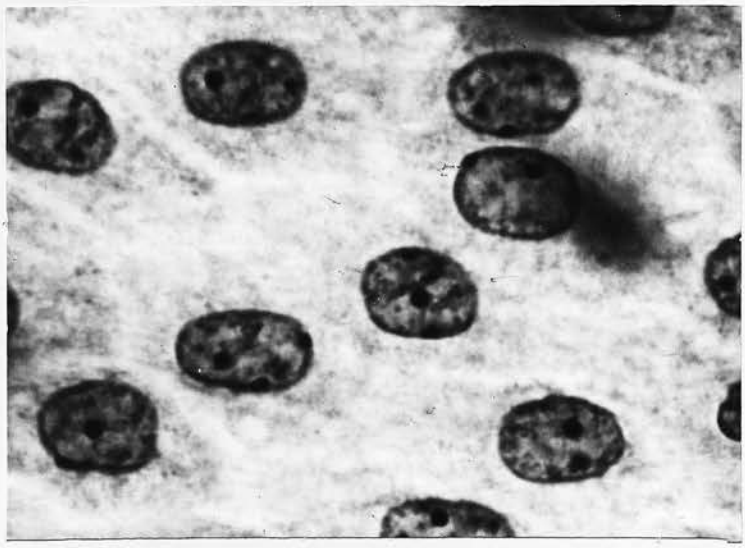

Fig. 107

Fig. I08

Monolayer of fibroblasts growing from a skin culture. x 45

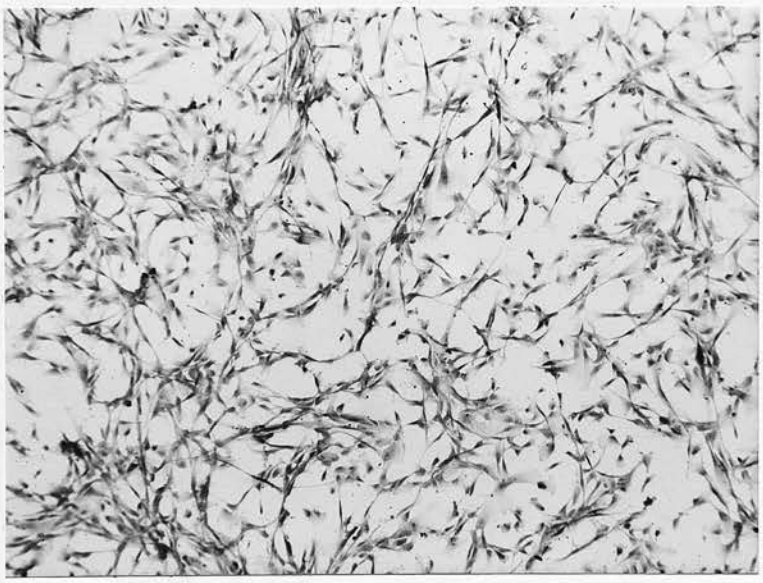

Fig. 108

Fig. 109, a & b

Chromosomes in a normal male cell nucleus, and the normal male karyogram.

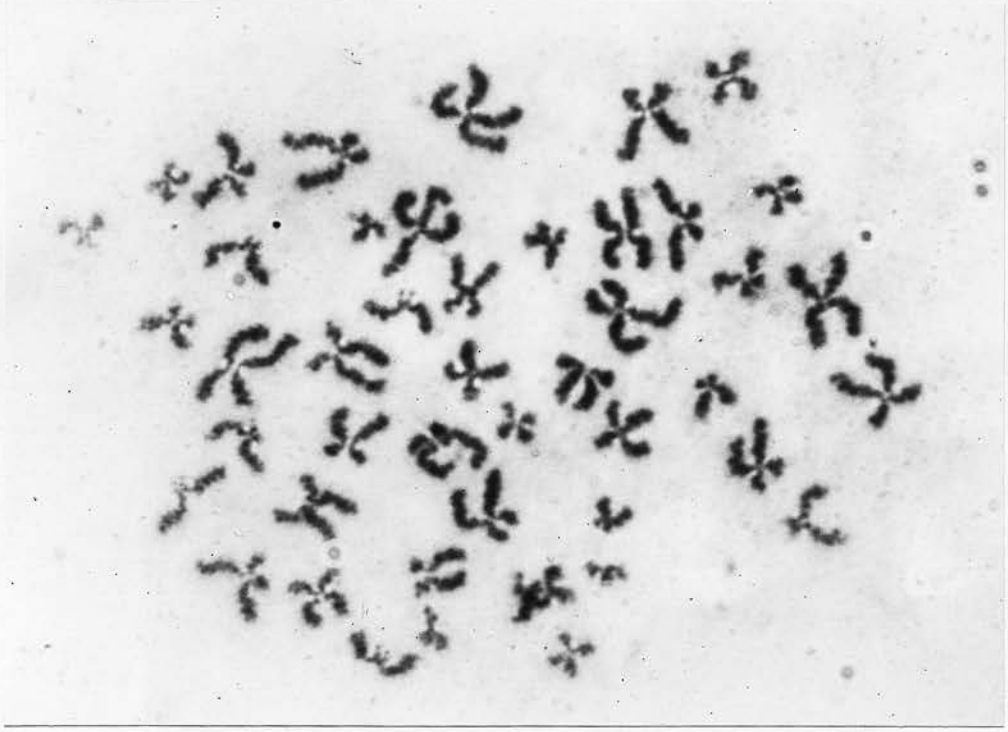

Fig. 109, a

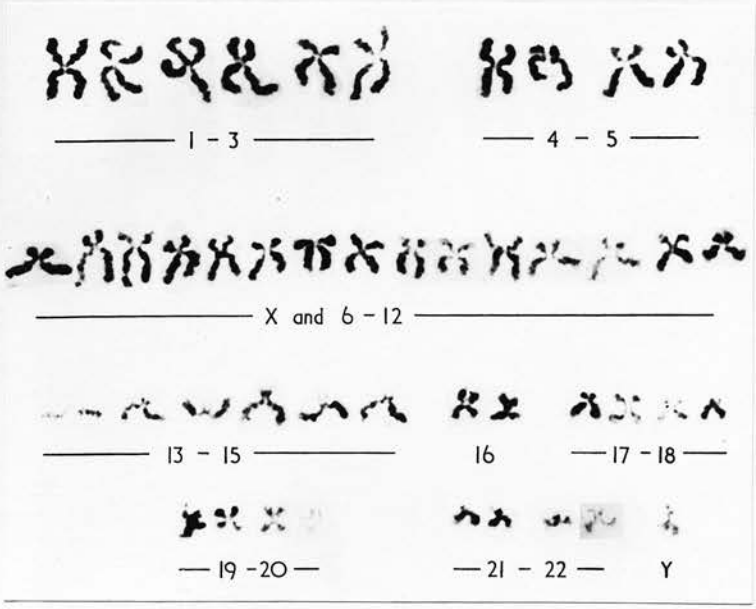

Fig. 109, b

Fig. IIO, a & b

Normal female chromosomes and normal female karyogram.

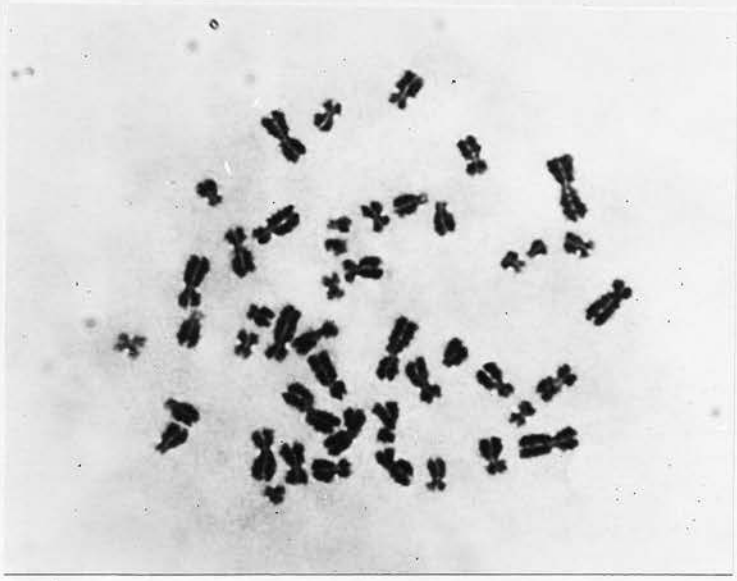

Fig. 110, a

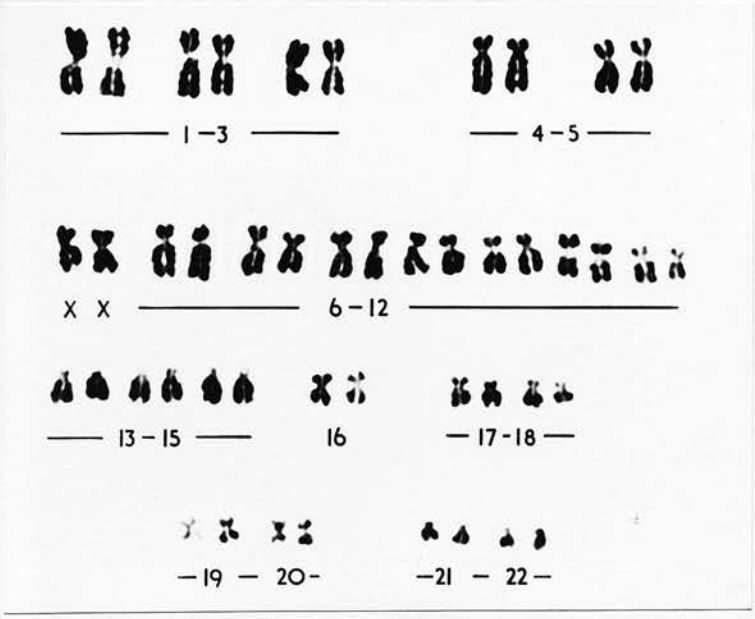

Fig. 110, b