CHAPTER 16
VIRGINITY, PREGNANCY AND DELIVERY

VIRGINITY

A virgin *(virgo intacta)* is a female who has not experienced sexual intercourse. **Defloration** means loss of virginity. The question of virginity arises in case of (1) nullity of marriage, (2) divorce, (3) defamation, and (4) rape.

Legally, **marriage** is a contract between man and woman, which implies physical union by coitus. **Divorce** means, dissolution of previously valid marriage.

It can be obtained on the ground of adultery, unnatural sex practices, desertion, cruelty and in cases of incurable insanity, leprosy or venereal diseases.

Nullity of marriage, i.e. marriage never to have existed in law. A marriage can be nullified: (1) When either party was under the age of marriage contract. (2) When one party was of unsound mind or a mental defective at the time of marriage. (3) When one party was already validly married. (4) Where the marriage has not been consummated due to impotence or wilful refusal. (5) Where the woman was pregnant by another man at the time of marriage.

Genitals : The labia majora are the two elongated folds of skin projecting downwards and backwards from the mons veneris. They meet in front in the anterior commissure and in back in the posterior commissure in front of the anus. In a virgin they are thick, firm, elastic and rounded, and lie in apposition so as to completely close the vaginal orifice. The labia minora (4 cm.long) are two thin folds of skin just within the labia minora. The lower portions of labia minora fuse in midline and form a fold called fourchette. The depression between fourchette and the vaginal orifice is called fossa navicularis. The labia minora are soft, small, pink, and sensitive. The clitoris is small and the vestibule is narrow. The vestibule is the triangular surface which extends from the clitoris above to the anterior margin of the hymen below, and laterally to the labia minora. It is usually concealed by the labia. Urethral opening is 2.5 cm. behind the clitoris, and immediately in front of vaginal opening. The posterior commissure and fourchette are intact. Fourchette is usually lacerated during child birth. Vulva includes the mons veneris (pad of fat lying in front of the pubis), labia majora and minora; clitoris, vestibule, hymen and urethral opening. The perineum is the wedgeshaped area between the lower end of posterior wall of vagina and the anterior anal wall.

The vaginal passage is a pocket irregular in shape, rather than cylindrical tube. It is about 7.5 cm. long, shorter on its anterior wall (6 cm.), and longer on the posterior wall (9 cm). It is collapsed to form a slit crosswise of the body. Distended it forms a gour-shaped balloon, wider at the top and possibly lopsided because of the greater size of one lateral pocket or fornix. The width at the upper end is 3 to 4 cm. in nullipara, and 6 to 7 cm. in the parous woman. Bladder lies anteriorly, levator ani muscles laterally, perineal body and rectum lies posteriorly, and pouch of Douglas superiorly to the vagina. The cervical canal is nearly at right angle to the vagina when bladder and rectum are empty. The vagina is narrow and tight, the mucosa is rugose, reddish in colour, sensitive to touch, and its walls are approximated. After frequent sexual intercourse (about fifteen to twenty sexual acts) the vaginal rugae become less marked, and the vagina lengthens into the posterior fornix, and the full length of the examining finger can be passed into the posterior fornix. The rugosity is removed only by first birth, but rarely it is absent even in a virgin. The hymen is intact. A single intercourse does not alter the parts much, except rupture of the hymen.

When a virgin is placed in lithotomy position with legs wide apart, the vagina remains closed and only the edges of labia minora are seen slightly protruding from between the closed labia majora. In women who have borne children labia are open and the vaginal canal is exposed.

Hymen: The hymen is a fold of mucous membrane about one mm. thick, situated at the vaginal outlet. The average adult hymen consists of folds of membrane having annular or crescentic shape, the broadest part lying posteriorly. The diameter of hymenal orifice in children is roughly

one mm. per year; more than one cm. diameter in a prepubertal child is seen commonly in abused girls. With repeated injury the posterior fourchette may develop an irregular thickened scar. Up to the age of 3 to 4 years the hymen is often annular, fleshy and fimbriated. From then until the onset of puberty it is usually a thin delicate almost translucent membrane with a width of tissue of about 4 mm at 6'O clock position. During puberty it becomes thickened often with fimbriated or wavy edge. The normal pre-adolescent hymen is essentially a two-dimensional structure located two to three cm. inside the vaginal introitus. In children, hymen appears as a tight membrane when the thighs are separated. At about puberty, hymen enlarges and gradually appears as a series of folds. The structure of hymen varies considerably. It may be a thin membrane, or it may be tough, fleshy or cartilaginous. The normal hymen may lie between these two extremes. The hymen may be a rigid, fibrous, unyielding structure, or it may consist of elastic tissue and be easily distensible. Recognisable, though not severe haemorrhage occurs, when hymen is ruptured. In infants, a small swab can be passed through the hymenal orifice into the vagina. At ten years of age, the tip of the small finger and at puberty one finger can usually be passed into the vagina. The different types of hymen are : (1) **Semilunar** or crescentic (commonest type) : the opening is placed anteriorly. Notches or clefts are seen at 10 and 11 clock position, which may be equal in size or more prominent on one side. (2) **Annular** : opening is oval and situated near the centre of the membrane. (3) **Infantile** : a small linear opening in the middle. (4) **Cribriform** : several openings. (5) **Vertical** : the opening is vertical. (6) **Septate:** two lateral openings occur side by side, separated partially or completely by thin strip of tissue. (7) **Imperforate:**

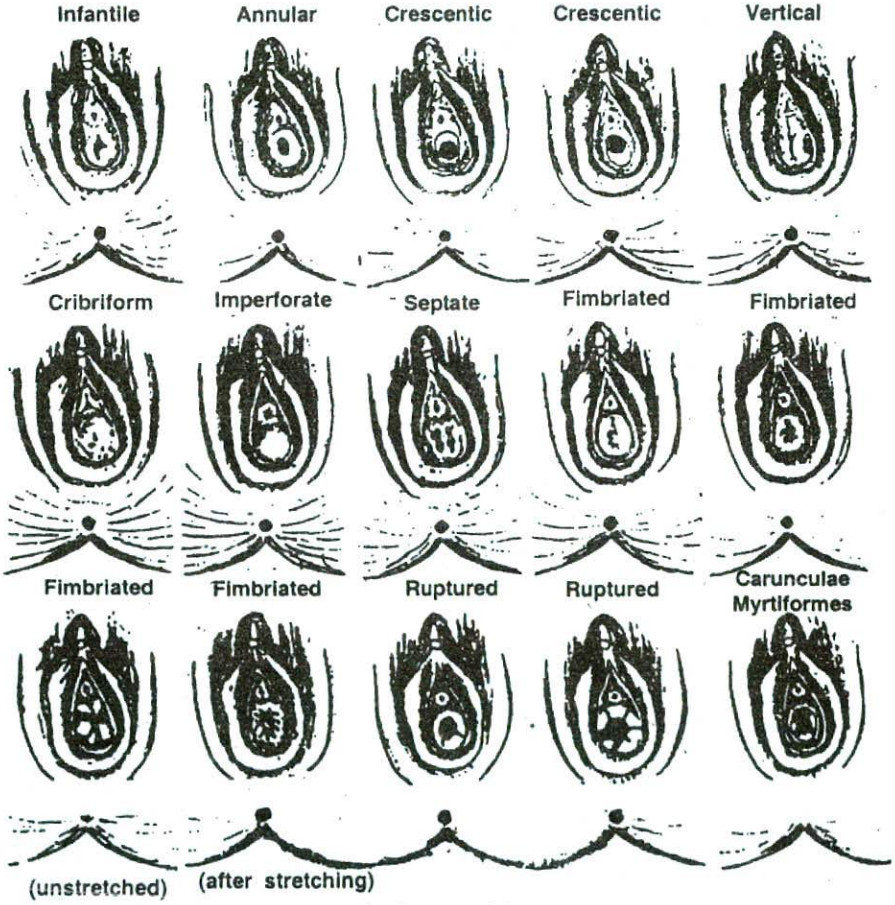

Fig. (16–1) Types of hymen.

Table (16–1) Difference between virginity and defloration.

Trait	Virginity	Defloration
(1) Hymen:	It is intact, rigid and inelastic; the edges are distinct and regular with a narrow opening hardly allowing a small finger to pass.	It may be torn or intact; in the latter case it is loose, elastic, with a wide opening allowing passage of two or more fingers.
(2) Labia majora:	They are adjacent to each other, fully developed and completely close the vaginal orifice.	They are not apposed to each other, not prominent and at the lower end vaginal orifice may be seen.
(3) Labia minora:	They are in contact and are covered by labia majora.	They are not in contact and are exposed and separated from labia majora.
(4) Fourchette :	Intact.	Torn or intact.
(5) Fossa navicularis :	Intact.	Disappears.
(6) Vagina :	It is narrow, the rugae more folded, and the vault more conical.	After repeated intercourse it usually grows in length, and the rugae are less obvious.

no opening.

The margin of the hymen is sometimes fimbriated (wavy or undulating) and shows multiple notches which may be mistaken for artificial tears. Natural notches are usually symmetrical, occur anteriorly, do not extend to the vaginal wall and are covered with mucous membrane. Tears caused by sexual intercourse or by foreign body are usually situated posteriorly at one or both sides, or in the midline, and usually extend to the vaginal wall and are not covered with mucous membrane.

Causes of Rupture of Hymen : (1) **An accident,** e.g., a fall on a projecting substance or by slipping on the furniture or fence or while playing at seesaw. In these cases tearing of the perineum occurs and usually injuries on other parts of the body will be seen. Such hymenal tears are never associated with abrasion and bruising of the margins. Accidental straddle injuries usually involve periurethral tissues, labia, hymen and mons. Separation of thighs forcibly in children will not rupture the hymen, unless perineum is ruptured. Hymen does not rupture by riding, jumping, dancing, etc. (2) **Masturbation,** especially with some large foreign body. Hymen is not injured in most cases because manipulation is usually limited to the parts anterior to the hymen. Labia minora and clitoris are enlarged in such cases. The vaginal orifice may be dilated and edges of the hymen may show scratches. (3) **Surgical operation** and gynaecological examination. (4) **Foreign body,** e.g., sola pith introduced into vagina for rendering very young girls fit for sexual intercourse (*aptae viris*). (5) **Ulceration** from diphtheria, fungus or other diseases. (6) **Scratching** due to irritation of the parts from lack of cleanliness. (7) Sanitary **tampon** may sometimes rupture the hymen.

Breasts: In a virgin the breasts are firm, elastic and hemispherical with a small undeveloped nipple surrounded by an areola which is pink in fair complexioned women and dark-brown in dark women. The breasts become large and flabby by frequent handling and sexual intercourse, but are not affected by single act of coitus. Occasionally, milk may be found in the breasts of virgins.

Medico-legal Aspects : The presence of unruptured hymen is a presumption, but is not an absolute proof of virginity. The diagnosis of virginity is difficult and in many cases a physical examination of the genital organs may not be helpful. With an intact hymen, there are true virgins and false virgins. The hymen is present always in a virgin in some form or other, but very rarely may be absent congenitally. The principal signs of virginity are : (1) An intact hymen. (2) A normal condition of the fourchette and posterior commissure. (3) A narrow vagina with rugose walls. These signs taken together, may be regarded as evidence of virginity but taken singly they cannot be so regarded.

The hymen is usually ruptured at the time of the first coitus, and at first only presents a torn appearance. Hymen may not be ruptured even after repeated acts of coitus if it is loose, folded and elastic, or thick, tough and fleshy, which permit displacement, distortion and stretching without

rupture. Cases have been recorded of women having sexual relations, of pregnant women and even prostitutes in whom the hymen was intact. In women who are used to coitus, and in those who have borne children, the hymen is destroyed and small, round, fleshy projections or tags, known as carunculae hymenales or myrtiformes are formed round the hymenal ring.

PREGNANCY

Pregnancy is the condition of having a developing embryo or foetus in the female, when an ovum is fertilized by a spermatozoon. It is most likely to occur between the ages of 14 and 45 years, but has been reported much earlier and later. The question of pregnancy has to be determined in the following conditions. (1) When a woman pleads pregnancy to avoid attendance in Court as a witness. Pregnancy itself is not an excuse, unless it is so far advanced that delivery is likely to occur soon, or when she or the child is likely to suffer risk by such attendance. (2) When a woman sentenced to death, pleads that she is pregnant, to avoid execution. The High Court has the power to postpone the execution of death sentence until 6 months after delivery or to commute it (S.416, Cr.P.C.). (3) When a woman feigns pregnancy soon after death of her husband to claim succession to estate. (4) To assess damages in a seduction or breach of promise of marriage case. (5) When a woman blackmails a man and accuses that she is pregnant by him, to compel marriage. (6) In allegations that an unmarried woman, widow or a wife living apart from her husband is pregnant. (7) When a woman alleges that she is pregnant in order to get greater compensation when her husband dies through the negligence of some person. (8) When pregnancy is alleged to be motive for suicide or murder of unmarried woman or widow. (9) In cases of divorce, the woman may claim to be pregnant to receive more alimony. (10) In cases of alleged concealment of birth or pregnancy and infanticide.

The written consent of the woman should be taken after explaining the reason for the examination and its possible consequences.

Diagnosis of Pregnancy: The signs and symptoms are usually classified into three groups: (1) The presumptive signs. (2) The probable signs.(3) The positive signs.

(I) Presumptive Signs : (1) Amenorrhoea: This is the earliest and one of the most important symptom of pregnancy. The periods may be missed for some time in unmarried woman after illicit intercourse simply from fear and nervousness. In married woman the periods may stop for some time, when there is an intense desire for pregnancy. Women who have never menstruated may become pregnant, and pregnancy may also occur in a woman during the amenorrhoea of lactation.

(2) Changes in Breasts : Breast changes are quite characteristic in primigravidas but are of less value in multiparas. A sense of tenseness and tingling in the breasts is frequent in early weeks. After the second month, breasts begin to increase in size and become nodular due to hypertrophy of the mammary alveoli. As they become still larger, the superficial veins are seen more distinct and enlarged, the nipples more deeply pigmented and more erectile and the areola which is pink in the virgin, gradually becomes dark-brown. Around the nipple, the sebaceous glands become enlarged by the end of second month to form small rounded dark coloured tubercles. (Montgomery's tubercles). **Colostrum** is secreted usually in the third month, which can be expressed from the breasts by gentle massage. Colostrum is thin, yellowish fluid consisting of fat globules and large phagocytic cells filled with droplets of fat. After six months, silvery lines or striae are seen especially in primiparae due to the stretching of the skin.

(3) Morning Sickness : It usually appears about the end of the first month and disappears 6 to 8 weeks later. Nausea and vomiting are usually present in the morning, and pass off in a few hours. It varies greatly in severity and is not reliable.

(4) Quickening : From about the 16th to 20th week, the pregnant woman feels slight fluttering movements in her abdomen, which gradually increase in intensity. These are due to movements of the foetus, and their first appearance is known as **"quickening".**

(5) Pigmentation of the Skin : The vulva, abdomen and axillae become darker due to the deposit of pigment, and a dark line extends from the pubis to beyond the umbilicus, the so-called **linea nigra.**

(6) Changes in the Vagina : The mucous membrane of the vagina changes from pink to violet, deepening to blue as a result of venous obstruction, after the fourth week. This is known as

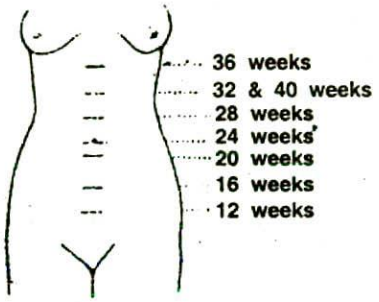

Fig. (16–2). Fundal heights during pregnancy.

Jackquemier's sign or Chadwick's sign. The anterior wall of the vagina is flattened. The tissues become softer, the secretion of the mucus is increased, and pulsation can be felt at an early period.

(7) Urinary Disturbances : During the early weeks of pregnancy, the enlarging uterus exerts pressure on the bladder and produces frequent micturition. This gradually disappears after few months, as the uterus rises up into abdomen, and reappears a few weeks before term when the head descends into the pelvis.

(8) Fatigue : Easy fatigue is very frequent.

(9) Sympathetic Disturbances : Salivation, perverted appetite and irritable temper are common.

(II) Probable Signs of Pregnancy :

(1) Enlargement of the Abdomen : During pregnancy, abdomen gradually enlarges in size after the twelfth week. By the end of third month, the uterus fills the pelvis, and between third and fourth months appears over the brim. At the fifth month, it is midway between the symphysis and umbilicus. At the end of sixth month, it is at the level of the umbilicus, and at the seventh month it is midway between the umbilicus and the xiphisternum, and at the end of the eighth month it reaches the xiphoid cartilage. During the last two months, the uterus sinks into pelvis and tends to fall forward due to its weight. The umbilicus becomes level with the skin by about the seventh month. Striae gravidarum are pinkish or slightly bluish, curved, irregular, depressed lines arranged more or less concentrically, sometimes radially around umbilicus, gradually becoming broader and deeper near inguinal ligament. They are seen in late pregnancy and around the time of delivery, become paler with time, and after a year or so appear as white scars (linea albicantes). They are caused by rupture of subcuticular elastic fibres, due to gradual distension of abdomen.

(2) Uterus : Hegar's sign is positive at about the sixth week. If one hand is placed on the abdomen and two fingers of other hand in the vagina, the firm hard cervix is felt and above it the elastic body of the uterus, while between the two the isthmus is felt as a soft compressible area. This is the most valuable physical sign of early pregnancy.

(3) Cervix: From the second month, the cervix progressively softens from below upward, which is well marked by fourth month. This is known as **Goodell's sign.** There is shortening of the cervix towards the last months of pregnancy. The orifice becomes circular instead of being transverse, and admits the point of finger to greater depth.

(4) Intermittent Uterine Contractions (Braxton-Hick's sign) : Intermittent, painless uterine contractions are difficult to be observed before the third month, but are easily felt after the fourth month. Each contraction lasts about a minute and relaxation for about two to three minutes. They are present even when the foetus is dead.

(5) Ballottement : It means to toss up like a ball. This is positive during the fourth and fifth months of pregnancy as the foetus is small in relation to the amount of amniotic fluid present. To obtain vaginal ballottement, two fingers are inserted into the anterior fornix and a sudden upward motion given. This causes the foetus to move up in the liquor amnii and after a moment, the foetus drops down on the fingers like a ball bouncing back. External ballottement can be obtained by imparting a sudden motion to the abdominal wall covering the uterus; in a few seconds the rebound of the foetus can be felt. This can be negative if the amniotic fluid is scanty.

(6) Uterine Souffle : This is a soft blowing murmur, which is synchronous with the mother's pulse. It is heard by auscultation on either side of the uterus just above inguinal ligament, towards the end of fourth month. It is due to passage of blood through the uterine vessels.

(7) Biological Tests : They are based on the reaction of test animals to chorionic gonadotropins contained in the pregnant woman's blood or urine. They are: (1) The rapid rat test. (2) The Aschheim–Zondek test. It is also positive in hydatidiform mole, chorionic epithelioma and ectopic pregnancy. (3) Freidman test. (4) Hogben or female toad test. (5) Male frog test. (6) Galli-Mainini test.

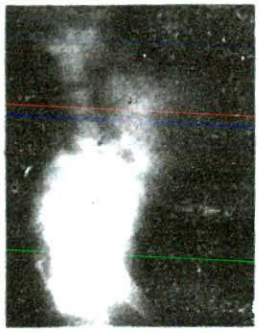

Fig. (16–3). Twin pregnancy

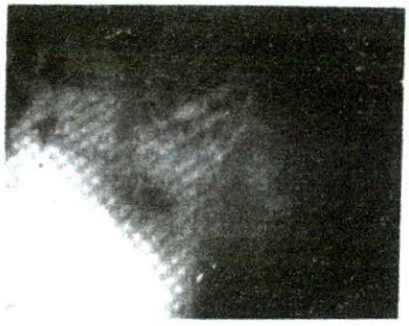

Fig. (16–4). X-ray photograph of the abdomen showing twin pregnancy. One foetus is dead and crumpled and shows Spalding's sign.

(8) Immunological Tests : The hormone chorionic gonadotropin (HCG) and human chorionic somatomamnotropin (HCS) are secreted by the syncytial trophoblastic cells into the fluids of the mother. It can be detected in maternal blood on about the eighth day after impregnation. The rate of secretion rises rapidly to reach a maximum about seven weeks after conception, and decreases to a relatively low volume by 16 weeks after conception. HCG is a glycoprotein and acts on same receptors as LH. These tests utilise antibodies to react with another substance for the detection of HCG. Because they are convenient and very reliable (accuracy 98%), they have replaced bioassays for routine screening. An early morning urine specimen will contain the highest level of HCG and is preferable for testing. Tests are positive 12 to 15 days after implantation.

(1) INHIBITION (INDIRECT) LATEX SLIDE TEST : A simple rapid test employs polysterene latex particles coated with a purified preparation of human chorionic gonadotropin as the antigen and antiserum to HCG. A drop of antiserum is mixed with a drop of urine on a glass slide for 30 seconds. Then, 2 drops of the sensitised latex particles are added and mixed with the combination of antiserum and urine. The slide is gently agitated for two minutes. If HCG is present in the urine it will combine with the anti-HCG antibody. This will leave no antibody free to combine with the latex HCG and there will be no agglutination of the latex particles (positive test). If there is no HCG in the urine, the antibody will be free to combine with the latex HCG and cause agglutination of the latex particles (negative test).

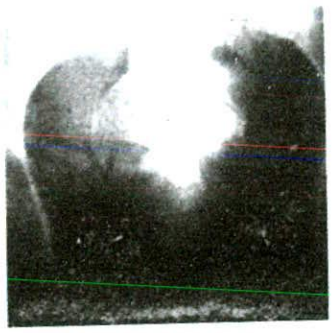

Fig. (16–5). X-ray photograph of the pelvis showing a dead foetus. The bones of the cranial vault show loss of alignment and overlapping (Spalding's sign).

(2) DIRECT LATEX SLIDE TEST : In this the latex reagent consists of particles coated with anti-HCG antibodies. This reagent is mixed directly with the urine. If HCG is present in the urine it will combine with the antibodies and cause agglutination of the latex particles (positive test). If no HCG is present in the urine, there will be no agglutination of the latex particles (negative test).

(3) Haemagglutination inhibition tube test.

Radio immunoassay and ELISA test can detect pregnancy with high degree of accuracy.

(III) Positive Signs of Pregnancy:

(1) Foetal Parts and Movements : Foetal movements are felt by placing the hands on the abdomen by 24 weeks. Foetal parts can be identified by abdominal palpation by 36 weeks.

(2) Foetal Heart Sounds : They are important and definite sign of pregnancy. They are heard between 18 to 20 weeks for the first time. The sounds are like the ticking of a watch placed under a pillow. Their rate is usually about 160 at fifth and 120 at the ninth month. They are not synchronous with the mother's pulse. Foetal heart sounds are not heard: (1) when the foetus is dead, (2) when there is excessive quantity of liquor amnii, (3) when

abdominal wall is very fat, (4) when examination is made before 18 weeks of pregnancy.

(3) X-ray Diagnosis : At about fifteen to sixteen weeks, foetal parts can be detected with certainty, but occasionally parts are detected as early as ten weeks. The shadows to be searched in the pelvis of the mother are: (1) Crescentic or annular shadows of the skull. (2) A series of small dots in a linear arrangement of the vertebral column. (3) A series of fine curved parallel lines of the ribs, and (4) Linear shadows of the limbs. Usually the skull and spine are seen at fifteen to sixteen weeks.

At a later stage X-ray examination may be of value in the diagnosis of a twin pregnancy, foetal death or abnormality, hydatidiform mole, etc.

Radiological signs of foetal death are : (1) Spalding's sign. (2) Collapse of the spinal column due to absence of muscle tone. (3) Presence of gas in the heart and great vessels.

(4) Sonography: This is done by employing three dimensional scanning with ultrasound. Gestational sac is seen as white ring by sixth week, and distinct echoes from the embryo within the gestational ring by seven weeks. Foetal heart beat can be made out by tenth week, and foetal head and thorax by fourteenth week. It can detect intrauterine growth retardation and foetal malformations.

Pseudocyesis (spurious or phantom pregnancy): It is usually observed in patients nearing the menopause or in younger women who intensely desire children. Most of the women suffer from some form of psychic or hormonal disorder. Such patients may present all the subjective symptoms of pregnancy associated with a considerable increase in the size of the abdomen which may be due to abnormal deposition of fat or to tympanites or occasionally due to ascites. Changes in breast are sometimes present and in many cases, the woman may imagine foetal movements. In some cases, pregnancy had gone to "full term" and frank labour pains occurred which ceased abruptly when the patients were told that they were not pregnant. Clinical examination (if necessary under anaesthesia) and X-ray examination will solve the problem. It is possible for a woman to be pregnant and not to know it. In rare cases pregnancy may progress to full term without the woman being aware of the fact.

Signs of Pregnancy in the Dead : The presence of an embryo, foetus, placental tissue, membranes or any other product of conception is a positive proof. The uterus is thickened and increased in size. A well-formed corpus luteum is found in one of the ovaries. Even in an exhumed body which has been reduced to a skeleton, foetal bones will be found in the remains.

The Period of Gestation :

(1) The Average Period : The usually accepted average is 280 days from the first day of the last menstrual period, so that the actual period of gestation is about 270 days or less. In most women, the period of gestation is forty weeks or ten times the normal intermenstrual period, which is usually 28 days.

(2) The Maximum Period : Mc Keown and Gibson have well documented cases up to 328 days, and from an extensive study have concluded that for medico-legal purpose, a period of 354 days from the coitus to livebirth is not impossible. The maximum period accepted by English Courts on medical evidence was 331 days. As a rule, the longer the gestation, the larger the infant.

(3) The Minimum Period and Viability: Children born at or after 210 days of uterine life are viable, i.e., are born alive and able to survive. Children born after 180 days of uterine life may be viable and capable of continuing an independent life apart from their mothers. Infants born at still shorter periods have sometimes survived and grown-up.

Posthumous Child : It is a child born after the death of its father, the mother being conceived by the said father. Legal issues involved are: legitimacy, inheritance of property, compensation case for slander against the mother.

SUPERFECUNDATION : It means the fertilisation of two ova which have been discharged from the ovary at the same period by two separate acts of coitus committed at short intervals. The incidence of twin pregnancies is about one-and-half%, and of these about 70% are binovular twins resulting from separate fertilisation of two ova formed in the same menstrual cycle. Development of twins in utero is parallel but not equal, depending on the relative blood supplies from the separately formed placentae. Both ova do not always develop to maturity. One foetus may be aborted early or die and retained until the labour that expels the other. The dead foetus may be flattened by pressure and may not be recognisable, and is referred to as **foetus compressus or foetus papyraceus**. The spermatozoa causing fertilisation may be from

different men. The rare cases wherein two ova are fertilised by a white and black person or persons with entirely different blood groups are the only certain examples of this condition.

SUPERFOETATION : This means the fertilisation of a second ovum in a woman who is already pregnant. Later, two foetuses are born either at the same time showing different stages of development, or two fully developed foetuses are born at different periods varying from one to three months. Cases where a second fully developed child was born a considerable time after the first have been explained on the assumption of twin pregnancy, in which the second child did not develop either due to diminished blood supply or some other cause. On the birth of the normally developed child, the second foetus received proper nourishment and is born later as mature child. Evidence is accumulating which indicates that superfoetation is not only a possibility but a reality. Its occurrence in a bipartite or double uterus is certainly possible.

THE PRE-NATAL DIAGNOSTIC TECHNIQUES (REGULATION AND PREVENTION OF MISUSE) ACT WAS AMENDED AND NOW IT STANDS AS THE PRECONCEPTION AND PRENATAL DIAGNOSTIC TECHNIQUES (PROHIBITION OF SEX SELECTION) ACT, 1994.

Chapter 1 deals with some definitions in context with the Act. Chapter II and III lay down certain conditions for regulation of various provisions of the Act. Some important conditions are furnished below:

Genetic Counselling Centres, Genetics Laboratories, Genetic Clinics, unless registered under the Act, cannot conduct or associate with, or help in, conducting activities relating to pre-natal diagnostic techniques. They cannot employ or cause to be employed or take services of any person whether on honorary basis or on payment who does not possess the prescribed qualifications.

Prenatal diagnostic procedures cannot be conducted unless (1) all side and after effects of such procedures have been explained to the concerned pregnant woman, (ii) age of the pregnant woman to be above thirty-five years and she has undergone two or more spontaneous abortions or foetal loss, (iii) pregnant woman had been exposed to potentially teratogenic agents, such as drugs, radiation, infection or chemicals, (iv) pregnant woman or her spouse has a family history of mental retardation or physical deformities, such as spasticity or any other genetic disease, (v) her written consent to undergo such procedures to be obtained in the language which she understands and a copy of her written consent to be given to the concerned pregnant woman, and (vi) communication of sex of the foetus by words, signs or in any other manner to the concerned pregnant woman or her relatives or any other person to be prohibited.

No Genetic Counselling Centres or Genetic Laboratories or Genetic Clinic or any person can conduct or cause to be conducted pre-natal diagnostic techniques including ultrasonography for the purpose of determining the sex of the foetus. No person can cause or allow to be caused, selection of sex before or after conception.

Chapter VII of the Act encompasses 'Offences and Penalties' as a result of violation of the provisions of the Act:

Advertisement relating to pre-conception and pre-natal determination of sex or sex selection is prohibited. Contravention is punishable with imprisonment up to three years and with fine up to ten thousand rupees.

Any medical geneticist, gynaecologist, registered medical practitioner or any person who owns a Genetic Counselling Centre, a Genetic Laboratory or a Genetic Clinic or is employed therein and renders his professional or technical services there, whether on an honorary basis or otherwise and who contravenes any of the provisions of the Act or rules made thereunder shall be punishable with imprisonment up to three years and with fine up to ten thousand rupees and on anay subsequent conviction, with imprisonment up to five years and with fine up to fifty thousand rupees. The name of the registered medical practitioner shall be reported to the State Medical Council concerned for taking necessary action.

LEGITIMACY AND PATERNITY

Legitimacy is the legal state of a person born in lawful marriage. If a person is born during the continuance of a legal marriage, or within 280 days after the dissolution of the marriage by divorce or death of the husband, his birth is presumed to be legitimate (S.112, I.E.A).

The child becomes illegitimate or **bastard**, if it can be proved that the husband could not possibly be the father of child by producing evidence that the alleged father is: (1) under the age of puberty, (2) physically incapable to beget children, because of illness or congenital or acquired deformities, (3) did not have access to his wife during the time that the child was begotten, (4) the blood groups of the child and the alleged father are not compatible.

The question of legitimacy arises in:

directed radially towards the anal canal. (8) Lesions are marked in children because of great disproportion in size between anal orifice of victim and the penis of the accused. If a moderate-sized penis is introduced without violence and with care, the anus may dilate sufficiently, and the act may be completed without leaving any trace. The anal sphicter of an infant admits one and in most cases two fingers without sustaining any injury. Signs of injury similar to those of penile penetration are produced if two or more fingers or large objects are introduced into the anus. (9) There may be anal prolapse. (10) Blood stains around the anus, on perineum and on clothes. (11) Faecal matter around the anus. (12) Lubricant matter, seminal fluid or venereal infections found at the anus or recovered by swabs from the rectum is a strong evidence of the crime. (13) There is pain during walking, defaecation and anal examination. (14) Signs of struggle, e.g., bruises and abrasions, if he is not a consenting party.

The smoothness of the anal margin skin, spasm of the anal sphincter, and the fresh appearance of abrasions may disappear in one to two days. A fissure remains visible for many days and a perianal haematoma takes seven to ten days for absorption. If the act has been performed carefully, or if the passive agent is habituated, there may be no signs as the anus can undergo considerable distension without injury if it is dilated slowly. In the living, a lax anus, prolapsed mucosal rim and thickened anal margin may be genuine signs. The only proof of sodomy is the presence of semen in the anus. Opinion as to the cause of the dilation should be guarded and it should only be stated that it is consistent with entry of a penis.

Habitual Passive Agent : The following signs may be present. (1) The shaving of the anal hair. (2) The skin about anus becomes smooth and thickened extending up into the anal canal to the mucocutaneous junction and sometimes into the upper anal canal. (3) The muscle of the anus loses its tone and does not contract so readily when the skin around it is pinched. (4) Slight depression of buttocks towards the anus due to absorption of fat. Funnel-shaped anus is very rare, and is usually an anatomical variant. (5) Dilatation and laxity of the anus, and an epithelisation of the wrinkled mucosa of this part. **Lateral buttock traction test:** A thumb is placed on each side of the anus and lateral traction is applied. In a habitual sodomite a complete relaxation of the sphincter occurs with dilation of the opening which may be four to five cm. in diameter through which rectum can be seen. Lax (overstretched) anus is significant; but elastic (capable of dilation) has no value. (6) There may be anal fissures, scars, etc. (7) Absence of fine wrinkles in the anal mucosa. (8) Piles and fissures are very common in old sodomists. (9) Presence of venereal disease.

Specimens to be Collected : (1) Blood. (2) Urine. (3) Head hair. (4) Pubic hair. (5) Loose hair and fibres found anywhere on the body. (6) Swabs from any soiled areas of skin. (7) Swabs from the anal, perianal and lower rectum. (8) Nail scrapings.

Active Agent : The routine medical examination of the accused of sodomy should follow similar pattern as in the case of alleged accused in rape. (1) The only evidence commonly found is the peculiar smell of anal glands transferred to the penis, and traces of faecal matter and lubricant on the organ. (2) Abrasions on the prepuce, glans penis or tearing of fraenum. (3) Faecal soiling, blood and foreign hairs are likely to be found in the area of coronal sulcus. (4) The urethral swab may show faecal material and organisms similar to those found on the anal verge swabs from the passive agent, which corroborate penetration. (5) Blood and seminal stains. (6) Presence of venereal disease. Smears should be taken from the external meatus after applying pressure on the undersurface of the penis along the urethra for gonococci. (7) Marks of violence on the body. (8) The clothes may show seminal stains or a mixture of semen and faeces. (9) In habitual sodomites, penis may be elongated and constricted at some distance from the glans with twisted urethra.

Swabs should be taken from shaft of penis, coronal sulcus and glans. Transmission of AIDS may occur after only a few sexual acts in homosexuals, eunuchs and prostitutes.

Gay clubs, i.e. association of homosexuals are present in some countries like U.K., U.S.A., etc.

BUCCAL COITUS : (Coitus per os or sin of Gomorrah). According to the Bible, this sin was common in a town called Gomorrah. In this type of sexual offence, the male organ is introduced into mouth, usually of a young child. Rarely, faint teeth marks and abrasions may be seen on the penis. Death may result from aspiration of semen or impaction of the penis in the hypopharynx. The

diagnosis is made by finding semen in the respiratory tract or stomach. Spermatozoa can be found in the mouth up to nine hours provided: (1) the victim has not cleaned the teeth nor taken a hot drink after the incident, and (2) careful swabbing has been done by rubbing around inside mouth, under tongue and gum margins. The victim's mouth is rinsed with distilled water, which can be expectorated into a sterile container, centrifuged and examined. It is a punishable under, S. 377, I.P.C.

The male prostitutes will submit to homosexual acts either as oral or anal inserters or receivers and even perform intercourse in the armpits (playing the bagpipes). The submammary fissures and intercrural folds are sometimes used.

TRIBADISM: Female homosexuality is known as tribadism or **lesbianism.** According to Greek mythology, women of Isle of Lesbos practised this perversion.

Sexual gratification of a woman is obtained by another woman by simple lip kissing, generalised body contact, deep kissing, manual manipulation of breasts and genitalia, genital apposition, friction of external genital organs, etc. In some cases enlarged clitoris is used as organ of passion or some artificial penis or phallus may be used. The external genitals may show scratch marks, abrasions or teeth marks. Many lesbians are masculine in type, possibly because of endocrine disturbances and are indifferent towards individuals of the opposite sex. The practice is usually indulged in by women who are mental degenerates or those who suffer from nymphomania (excessive sexual desire). It is the result of interactions of biological, psychological, developmental and sociologic factors. It may lead to interference with young girls. Lesbians who are morbidly jealous of one another, when rejected may commit homicide, suicide or both. Tribadism is not an offence in India.

BESTIALITY: Bestiality is the sexual intercourse by a human being with a lower animal. The animals involved include those that are kept on the farm or as pets in households. Because of their convenient size, animals like calves and sheep are more often involved. A few of larger birds like chicken, ducks and geese are also involved. Other animals used are cows, mares, she-asses and bitches. Vaginal intercourse is the most common, but intercourse may take place through the anus or any other orifice, e.g., nose. This is seen in persons suffering from mental abnormality. Persons who go out to graze cattle in the fields may be excited when alone with the animals. Sometimes, the act is committed due to the false belief that gonorrhoea is cured by intercourse with a she-ass. Dogs and cats are the common animals for females. Usually the animal manipulates the genitalia with its mouth, and actual coitus is very rare. Both the accused and the alleged animal are to be examined.

Signs in the Accused: (1) Animal faeces, vaginal secretion or hair may be present on the penis. There may be tearing of the fraenum. (2) Marks of injuries on the body due to kicks, teeth or claws of the animal. (3) Presence of animal hairs especially of its external genitals on the person or the clothes. (4) Stains of dung or animal blood on the person or clothes.

Signs in the Animal: (1) Presence of human spermatozoa in the vagina or anal canal of the animal is a positive sign. (2) Abrasions and lacerations with effusion of blood on the external genitals. (3) Presence of gonorrhoeal discharges in the animal. It is better that the animal is examined by a veterinary surgeon.

Female Circumcision: Sexual mutilation of small girls is done in some African countries involving excision of prepuce of clitoris, clitoris, labia minora or any combination of three.

Priapism is persistent abnormal erection of the penis, usually without sexual desire, and accompanied by pain and tenderness. It is seen in diseases and injuries of the spinal cord and certain injuries to the penis.

SEXUAL PERVERSIONS

Sexual perversions (sexual deviations) are persistently indulged sexual acts or fantasies in which complete satisfacion is sought and obtained without sexual intercourse. The perverted behaviour is really a repulsively abnormal and frequently cruel form of tension release that by its nature tends to operate under compulsion. Most cases of sexual perversion involve early conditioning influences beyond the range of immediate knowledge. The mental state of the accused should be determined in all such cases by a psychiatrist.

URANISM: It is a general term for sexual perversion which includes sexual gratification by fingering, fondling, licking, etc.

PARAPHILIAS: Abnormal and unorthodox sexplay by using objects or parts of the body are

known as paraphilias.

SADISM: (algolagnia): The term is derived from the name of a French nobleman, the Marquis de Sade, infamous for his crimes and writings. Many of his stories were about sexuality, cruelty, and torture. In sadism, sexual gratification is obtained or increased from acts of physical cruelty or infliction of pain upon one's partner. It is seen more commonly in men. To obtain sexual gratification the sadist may bite, beat, whip, produce cuts, etc., or ill-treat or torture his sexual partner in many other cruel ways. Extremely sadistic attacks may be made in which the victim's nipples may be bitten off, articles such as a bottle, candle or sticks are inserted into the vagina, cigarettes or lighters may be used to burn the skin, or blows which may rupture internal organs or cause fractures. It develops due to early experiences of brutality in relation to sex. Many are sociopathic, some schizoid and others inadequate personalities.

LUST MURDER : In extreme cases of sadism, murder serves as a stimulus for the sexual act and becomes the equivalent of coitus, the act being accompanied by erection, ejaculation and orgasm. The typical lust murder is characterised by: (1) Periodic outbreaks, due to the patient's recurring compulsion or sudden outbursts of sexual desire. (2) Cutting or stabbing of the breasts, genitalia or the lower abdomen, usually with sucking, licking or mouthing of the wounds and biting of the skin. In some cases, there is desire to drink the blood and eat the flesh. (3) Erection and ejaculation may sometimes be followed by sexual intercourse with the dying or injured victim. (4) His behaviour is usually normal until the next outbreak. Many rape murders result from an aggressive sexual reaction to inner fear. In true lust murders, mental disease is quite frequent. Every murder committed during a sexual act is not a lust murder. The murder may be the result of anger, jealousy, revenge, etc.

NECROPHAGIA: (necros = corpse; phagia = to eat): This is extreme degree of sadism in which the person after mutilating the body, sucks or licks the wounds, bites the skin, drinks the blood and eats the flesh of his victim to derive sexual pleasure.

MASOCHISM: This term is derived from the name of Leopold von Sacher-masoch, an Austrian novelist. Being whipped by his wife used to be a stimulant for his literary work. This condition is the opposite of sadism. In masochism, sexual gratification is obtained or increased by the suffering of pain. Masochists get pleasure from being beaten, abused, tortured, humiliated, enslaved, degraded or dominated by their sexual partner, and they tend to place themselves repeatedly in self-defeating situations. Such painful stimuli may entirely replace the ordinary sex stimuli. It is usually found in males but it may be found in females, who may willingly expose themselves to the risks of severe bodily injury or murder at the hands of brutal husbands or lovers.

Sadism and masochism are rarely found in a pure state. They are usually found as a combination with one type dominant over the other. The combining of these practices is called **bondage.** They are found in all age groups and in all socio-economic levels. The acts of cruelty or pain associated with sado-masochism may serve as a stimulant for, or as a complete substitute for sexual intercourse.

Algolagnia includes both sadism and masochism.

NECROPHILIA: (philia = to defile or foul). In this condition, there is a desire for sexual intercourse with dead bodies. It is said to have sado-masochistic foundation and that decomposition, foul smell, and coldness act as stimulants. There is also no danger of rejection or resistance. The offence is usually committed on a newly buried corpse or a body awaiting burial. The corpse may be mutilated following intercourse. Murder for the purpose of necrophilia is very rare. Necrophilia and necrophagia are punishable under S. 297, I.P.C. with imprisonment up to one year.

FETICHISM: A fetish is an abnormal stimulus or object of sexual desire. Fetichism means the use of such objects for sexual gratification. In this, the person experiences sexual excitement leading to orgasm from part of the body of a woman or some article belonging to her that normally has no sexual influence on the mind, e.g., underclothing, brassiere, petticoat, stocking, shoes, etc. which act as substitute for the female love object. The fetish may be only incidentally associated with human body, e.g., a flower. In some cases, a picture of the fetish object provides sufficient stimulus. Sometimes, the act of stealing the articles provides adequate sexual satisfaction, though often the fetish article is stored to the satisfaction of the fetish, or touching it gives him sex pleasure, or he may masturbate into the object. It is almost exclusively seen in males. It is harmless, but rarely it may drive the person to obtain his fetish object through violence, or other

criminal act, e.g., objects may be stolen, or women may be attacked either as part of robbery with violence, or because the fetish provides the trigger for rape or indecent assault.

TRANSVESTISM OR EONISM : A transvestite (trans = opposite; vesta = clothing) is a person whose whole personality is dominated by the desire to be identified with the opposite sex. The term is derived from the name of Chevelier d' Eon Beamont, a Frenchman, who preached this. He wants to be thought of as a member of the opposite sex. His dress, manner, occupational interests and associations are all designed to increase his feeling of being a woman. Sexuality with him is relatively unimportant except as it promotes his feelings of feminity. There are varying degrees of transvestism. It is usually found in the males who obtain sexual pleasure by wearing female dress. Psychologically, it may depend upon an individual's erotic attraction for opposite sex. Only small percentage are homosexuals. Rarely, it develops out of a fetishistic interest in clothing or some part of opposite sex. Many cases are associated with sadomasochism. There is no hormonal disturbance or genital abnormality.

Scatalogia is associated with obscene telephone calls.

Sexual Oralism: It is the obtaining of sexual pleasure from the application of the mouth to the sexual organs. It is seen both in heterosexuals and homosexuals. **Fellatio** (blow job) is the oral stimulation or manipulation of the penis, either by the female or male. **Cunnilingus** (mouth job) is the oral stimulation of the female genitalia.

MASTURBATION: Masturbation (onanism: ipsation) is the deliberate self-stimulation which effects sexual arousal. Mild masturbatory exercises are common both to men and women and are of little importance. Techniques are largely manual, by moving the penis against a bed or other object. Urethral insertions and anal stimulation and anal insertions are rare. Hollow articles like bottles, test tubes, etc., are sometimes used, or articles made of rubber and plastic which simulate the female genitalia are used.

In females, a finger is gently and rhythmically moved over clitoris or labia minora or steady pressure is applied over these parts with several fingers or whole hand. The genitalia may be rubbed against a pillow, a bed or some other object. Sometimes, women may insert fingers, wooden rods, glass tubes, metallic bars, bananas, etc., or artificial masculine genital parts (dildo) made of rubber or plastic into the vagina. Masturbation is an offence only when practised openly, e.g., in telephone booths, lavatories, etc.

EXHIBITIONISM: It is a wilful and intentional exposure of the genitalia in a public place while in the presence of others, to obtain sexual pleasure. It may or may not be accompanied by masturbatory acts. It is done mostly by males, often to children or the persons of the opposite sex. The pervert adopts a childish method of attracting attention to himself, to experience sexual gratification at the time of the exhibition, without physical contact. In some cases, the act is premeditated. Occasionally, women may expose themselves in public. Majority of them are psychopathic or suffer from compulsion neurosis and suffer from alcoholism, epilepsy, senile dementia, GPI, etc. It is an obscene act punishable under S. 294, I.P.C. with imprisonment up to three months or fine.

VOYEURISM OR SCOPTOPHILIA: It is the counterpart of exhibitionism. The voyeur (so-called peeping Tom) must see people undress in order to be sexually satisfied. The perversion is in the sexual dependence upon "looking", "peeping", "seeing". There is a morbid desire to look at the sexual organs or other usually clothed parts of the body of one of the opposite sex, or to watch sexual intercourse as a source of sexual satisfaction. The act of observation can also result in attempts at exhibitionism or masturbation. This perversion occurs in case of severe sociopathic personality disorder. To many males, observation of a female who is undressing may be erotically more stimulating than observing her when she is fully nude. Usually such persons do not commit a major sex crime, but sometimes they may assault the victim, or commit a murder. It is rare in females.

TROILISM: It is sexual practice involving 3 persons, 2 of one sex and one of the opposite sex.It is an extreme degree of voyeurism. A perverted husband gets sexual satisfaction inducing his wife to sexual intercourse with another man and by watching the same.

MIXOSCOPIA: It is a form of voyeurism in which sexual gratification is obtained by the sight of others engaged in sexual intercourse.

OEDIPUS COMPLEX: It is sexual desire of son towards his mother. **Electra complex** is sexual desire of daughter towards her

father.Such persons are psychopaths with history of some mental trauma in the early life.

FROTTEURISM: Frotteurism is contact with another person in order to obtain sexual satisfaction. Sexual satisfaction is obtained by rubbing his private parts against a female's body in crowds. If they attempt intercourse they have a premature ejaculation or they are impotent. It is an uncommon perversion and rarely occurs alone. It is punishable under S. 290, I.P.C with fine up to 200 rupees.

UNDINISM: In this the sexual pleasure is often obtained by witnessing the act of urination by some one of the same or opposite sex. In some cases pleasure is obtained by being urinated upon by the loved one or in urinating on him or her, but this is rare.

PYROMANIA: In most cases of pyromania, there is an underlying psychosexual disorder. Some have a latent form of sadism, and others obtain sexual stimulation or satisfaction while seeing the flames and destruction of a building.

Caprolagnia: Sexual excitement is associated with sight or smell of faeces or defaecation.

Urolagnia: Sexual excitement is associated with the sight or thought of urine or urination.

Narcism (Narcissim): Self-love, which may or may not include genital excitation.

Pygmalionism: It is falling in love with an object made by him.

INDECENT ASSAULT: Indecent assault is any offence committed on a female with the intention or knowledge to outrage her modesty. Usually the act involves the sexual parts of either, or is sexually flavoured. In such assaults, a man may try to kiss a woman, press or fondle her breasts, touch or expose the genitalia or thighs, try to put a finger in her vagina, play with vulva, etc. This is usually committed against children, or adolescent girls and rarely on adult or old women. Stripping naked a female patient for medical examination is regarded as an assault. Men may encourage children to handle or masturbate their sexual organs. Indecent offences between two or more male persons include such offences as friction of penis on the gluteal folds, handling of the male genitalia, mutual masturbation, etc. or intercrural connection (penile friction between the inner thighs and external genitalia). Such assaults are punishable under S. 354, I.P.C., up to two years imprisonment and / or fine. In such cases, medical examination is of little value. Abrasions or bruises may sometimes be present due to struggle.

Whoever, intending to insult the modesty of any woman, utters any word, makes any sound or gesture, or exhibits any object shall be punished with imprisonment which may extend to one year (S.509, I.P.C.).

SEMINAL FLUID

Seminal stains have to be detected in cases of rape or attempted rape, sexual murder of the female, sodomy and bestiality. Fertility of the liquid has to be proved in civil cases, e.g., disputed paternity. Semen is greyish-yellow, thick, jelly- like and sticky when fresh, and has a characteristic odour. The quantity of seminal fluid in a single emission is two to five ml. and contains about 60 to 150 million sperms per ml.,of which 90 percent are motile at the time of ejaculation. Spermatozoa constitute about ten percent of the volume of the semen. Spermatozoa contain lipids, proteins and a number of enzymes. The morphology of spermatozoa is different even among closely related species. The fluid is alkaline with a pH of 7.4.

The stains are usually found on the clothing, but may be found on the person of either the victim or the accused. They may also be found on bed clothes, on floor or on the grass where the offence was committed. Seminal stains have to be differentiated from those due to starch, pus, leucorrhoeal discharge and egg albumen.

Collection of Material : (1) Fluid from the vagina is collected with a pipette or throat swab inserted with or without the aid of a speculum, or vaginal washing is done, which is concentrated by centrifugation. (2) Dried or drying seminal fluid on the perineum or thighs is collected with a wet throat swab. (3) A portion of cloth containing the stain is cut out, dried and preserved. (4) The pubic hair should be plucked and placed in a small container.

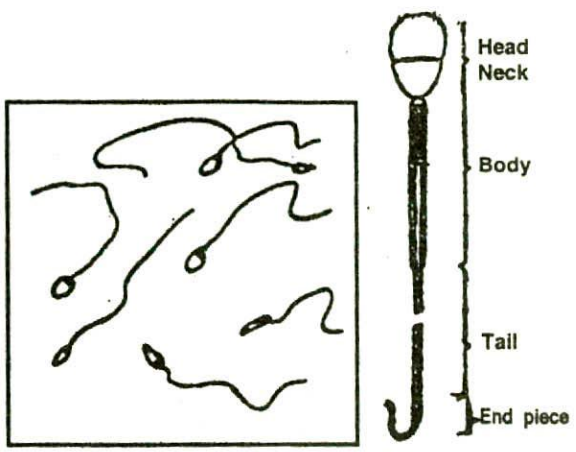

Fig. (17–3). Human spermatozoa.

(5) Stains on smooth, impervious surface should be gently scraped off with the point of a knife into a glass container.

(I) PHYSICAL EXAMINATION : Seminal stains when dry have a greyish-white or yellow-grey colour and show an irregular, map-like outline. The cloth is stiffened as if starched. A fresh stain on a non-absorbent material appears translucent. After a month it becomes yellow to brown. When examined under filtered ultraviolet light, they show a fluorescence of a bluish-white colour, which is not specific as other albuminous materials such as nasal, leucorrhoeal discharges and detergents also fluoresce. Fluorescence depends on choline of semen. It is masked by blood and disappears if the stain is soaked in water. Fresh stains have a characteristic odour.

(II) Chemical Examination :

(1) Florence Test : The stain is extracted by 10% hydrochloric acid, and a drop is placed on a glass slide and allowed to dry. A coverslip is placed over this, and a drop of Florence solution (potassium iodide, iodine, and water) is allowed to run under the coverslip. If semen is present, dark brown crystals of choline iodide appear immediately. They are rhombic crystals resembling haemin but are larger, arranged in clusters, rosettes, crosses, etc. Choline originates from the seminal vesicles. The test is not proof of seminal fluid, but only of presence of some vegetable or animal substance. A negative reaction is proof that the stain is not seminal.

(2) Barberio's Test : A saturated aqueous or alcoholic solution of picric acid when added to spermatic fluid produces yellow needle-shaped rhombic crystals of spermine picrate. The reaction probably depends on the presence of prostatic secretion.

(3) The Acid Phosphatase Test : The prostatic secretion element of seminal fluid contains 500 to 1000 times greater acid phosphatase than any other body fluid. Human red cells, semen of higher apes, and juice of cauliflower have acid phosphatase level similar to that of human semen. Undiluted semen has an acid phosphatase activity of 340 to 360 Bodansky units or 2500 to 3500 Angstrom units per ml. The concentration of acid phosphatase gradually falls with time in vaginal secretions, and positive reactions are found for periods of thirty-six hours, with gradual disappearance in 72 hours, but is little changed if the body is refrigerated. Five to ten ml. of normal saline solution is placed in the vagina with a syringe. The fluid is then removed and placed in a sealed tube and refrigerated for enzyme examination. Concentration in excess of hundred Bodansky units with or without motile sperms indicate that ejaculation occurred within twelve hours of examination. Dried seminal stains which have not undergone putrefaction retain acid phosphatase activity for weeks or months, although enzymatic activity decreases slowly with time. The concentration is slowly reduced when the stain is left at room temperature and exposed to light. Heating of the specimen to 60°C. or over destroys it within five minutes. In humans, acid phosphatase content is greater than in animals. This test is conclusive in the absence of demonstrable sperms or in aspermia.

(4) Creatine Phosphokinase : Spermatozoa contain a high concentration of creatine phosphokinase, which is more than double, than found in any other body fluid. Normal seminal fluid contains 385 to 1400 units of CPK/ml. Levels over 400 are almost diagnostic of seminal stains. The enzyme is stable and can be demonstrated even in old stains of six months.

(5) Immunological Method: MHS-5 produced from seminal vesicles and Mab 4 E6 can be detected on the sperm cells and in the ejaculated fluid. Prostate specific antigen (PSA, P30) is a glycoprotein produced by prostate and is found in seminal plasma (normal and aspermic semen), male urine and blood, but not in any tissues or fluid of the female. It is found in vaginal fluid up to twenty-seven hours after sexual intercourse, but sometimes may be detected up to forty-seven hours. Tests for P_{30} have replaced the quantitative analysis for acid phosphatase.

Fluorescent in situ hybridisation (FISH) has been suggested as a sensitive and specific test for detection of male epithelial cells in the post-coital vagina up to one week. Y-chromosome positive epithelial cells have been identified in vaginal swabs even in cases with no ejaculation.

(6) CHOLINE AND SPERMINE TEST : Liquid semen and dried seminal stains can be identified by a thin layer chromatographic technique. The test is based on the unique combination of choline and spermine which is present only in semen. Spermine is found in testes, pancreas, liver, spleen, brain, blood, bone marrow and body fluids, but its concentration is lower than in semen. One microlitre of semen present can be detected by this method.

(7) AMMONIUM MOLYBDATE TEST :To extract of the stain, ammonium molybdate reagent is added. A deep yellow colour from the presence of phosphorus is positive.

Other substances which have been advocated as a test for semen are sperm specific lactate dehydrogenase isoenzyme, peptidase A, phosphoglucomutase, and glyoxalase 1.

Methods for analysis of seminal stains include gel diffusion precipitate reactions, immuno-diffusion and isoelectric focusing.

Concentration of zinc in semen is 140 mg/ml whereas blood serum contains 1.2 mg/ml. It does not degrade even in old stains.

(3) Microscopic Examination : A small piece of the stained fabric is moistened with a few drops of one percent hydrochloric acid or three percent acetic acid in a watch glass for half to one hour when the stains are fresh or two to four hours when old. The central portion of the stain usually contains the largest number of sperms. Slides are prepared by rubbing the piece of fabric on them. Films are dried in the air without heat or fixed for one minute in methyl or ethyl alcohol and then stained. The slide is stained with methylene blue for 15 to 30 minutes and counterstained with eosin for two minutes. The posterior half to one-third of head is stained deep-red or pink, while the anterior half or two-third appears unstained or is faintly stained with basic dye. Alternatively, staining with haemalum two to five minutes, and eosin two to five minutes is satisfactory. The tail is stained pink. Old stains (even several years) may give positive results, but the older they are, the less is the chance of finding intact sperms. Complete sperms have been found on clean cotton material after five years. In the vagina, sperms are continually removed by phagocytosis, lysis, agglutination or degeneration as a result of local physico-chemical reactions. The slide should be etched with a glass marking pencil. A drop of mucus removed from vagina is placed on a glass slide, and diluted with a drop of normal saline and examined for motile spermatozoa. Human spermatozoa vary from 50 to 55 microns in length and consist of head, neck and tail. The head is oval and flattened when seen in front and pear-shaped in profile. It is five microns in length and three-and-half microns in its greater diameter. The neck is very short. The tail is long and tapered to a fine point. Sperms of various animal species differ in their morphology.

Spermatozoa are easily found in stains that dry rapidly. The head resists decomposition for some time, and as such, few heads of spermatozoa may be found in decomposed stain. They undergo disintegration within a few months, but may be found up to five years. With an ultrasonic apparatus, maximum recovery of complete spermatozoa is readily obtained.

Motility of Sperms : When semen is kept at room temperature, full motility persists for about three hours; fifty percent are motile by eight hours, and ten percent for 24 hours. If the specimen is kept at body temperature, motility persists for several hours. In living persons, motile sperms are usually seen up to six hours and rarely 12 hours after ejaculation into the vagina. Complete sperms are seen up to twenty-six hours, and occasionally up to two to three days. Willot and Allard (1982) state that vaginal spermatozoa may be found up to 120 hours after intercourse. Then they separate into heads and tails. In the dead, sperms are destroyed by decomposition, but not by drainage or the action of the vaginal secretion. Willmott (1975) reviewed the literature and reported that sperm head had been found in the vagina up to nine days and in the cervix up to twelve days. In the anus they have been found up to two days, and in the mouth up to nine hours after intercourse. Spermatozoa may be found up to a fortnight in the uterine cavity.

Precipitin Test : The principle and the technique is the same as that for blood.

Group of Seminal Fluid : The semen of the secretors contain ABO groups, PGM, AK, HLA, LDH, Gm, Km and GLO.

PROOF OF SEMEN : The only absolute proof of semen is the finding of at least one unbroken spermatozoon, or electrophoretic LHD isoenzyme detection of sperms. Positive identification of a sperm should not be made only on the basis of recognising "sperm head". In the absence of spermatozoa, a stain which gives characteristic fluorescence in ultraviolet light, positive precipitin test, high level of acid phosphatase and a high creatine phosphokinase and p30 can be considered to be due to semen.

In vaginal smear, bacteria, fungi, trichomonas, yeast, monilia, naked nuclei from vaginal epithelial cells and foreign substances are usually present, which may obscure or simulate sperm head or even entire spermatozoa. If there is any doubt whether some object in the smear is a sperm, the probability is that it is not.

Single photon fluorimetry has been used to differentiate between different semens. DNA can separate and identify different semens.

CHAPTER 18
ABORTION

Legally, abortion (miscarriage) means the premature expulsion of the foetus from the mother's womb at any time of pregnancy, before full term of pregnancy is completed.

Classification : (1) Natural : (a) Spontaneous. (b) Accidental. **(2) Artificial :** (a) Justifiable. (b) Criminal.

Natural Abortion : Abortion may occur at any time due to natural causes. Abortion occurs in 10 to 15% of all pregnancies and is most common about the second or third month. Within the first few weeks, ovum may be passed off without being recognised. In abortions during the first two months of pregnancy, the ovum is expelled intact, covered by the decidua vera. In later months, the foetus is born first, followed by the amniotic sac and placenta, and later on the decidual tissues. The products are expelled days or weeks after the death of the embryo.

Causes: (1) Defect in the ova, including chromosomal defect. (2) Developmental defect of the foetus (common cause). (3) Low implantation of zygote. (4) Disease of decidua or placenta. (5) Rh incompatibility. (6) Retroverted uterus. (7) Submucous uterine fibroid. (8) Malformed uterus. (9) Uterine hypoplasia. (10) Hypertension. (11) Diabetes mellitus. (12) Hormonal deficiency. (13) Sudden shock, emotional disturbances. (14) Syphilis. (15) Nephritis. (16) Arsenic or lead toxicity. (17) Drug toxicity.

Justifiable or Therapeutic Abortion : Abortion is justifiable only when it is done in good faith to save the life of the woman, if it is materially endangered by the continuance of pregnancy. The World Medical Association, adopted a resolution on therapeutic abortion, known as **Declaration of Oslo (1970).**

Criminal Abortion : A criminal abortion is the induced destruction and expulsion of the foetus from womb of the mother unlawfully, i.e., when there is no therapeutic indication for the operation. It is resorted to mostly by widows and unmarried women. It is usually carried out before the third month. A case of criminal abortion is investigated only when the woman dies, and rarely when some one gives the information to the police.

Unsafe abortion means, abortion not provided through approved facilities, and/or persons.

ABORTIONISTS : They can be divided into three main groups: (1) The expert or medically qualified abortionist. (2) The semi-skilled abortionist, such as midwives, nurses, chemists, etc., who have a fair knowledge of anatomy and pregnancy but who have not the facilities available to the qualified person. (3) The unskilled abortionist.

LEGAL ASPECTS : Under Sec. 312, I.P.C. whoever voluntarily causes criminal abortion is liable for imprisonment up to three years, and/or fine; and if the woman is quick with child the imprisonment may extend up to seven years. It is necessary that the woman should be pregnant and that abortion should be carried with her consent. Both the person causing the abortion and the woman are liable for punishment. If the means used do not succeed, it is punishable under Sec.511, I.P.C. with imprisonment up to half of the punishment under Sec. 312. Under Sec. 313, if the miscarriage is caused without the consent of the woman, the imprisonment may be up to ten years. Under Sec. 314, if a pregnant woman dies from an act intended to cause miscarriage, the offender is liable to be punished with imprisonment up to ten years. Under Sec. 315, a person doing an act intended to prevent the child from being born alive or to cause to die after its brith, is liable to be punished with imprisonment up to ten years. Under Sec. 316, causing death of quick unborn child by any act amounts to culpable homicide, and the punishment may extend up to ten years imprisonment.

THE MEDICAL TERMINATION OF PREGNANCY ACT, 1971

Indications: Under this Act, pregnancy can be terminated under the following conditions. **(1) Therapeutic** : When the continuation of pregnancy endangers the life of woman or may cause serious injury to her physical or mental health. **(2) Eugenic:** When there is risk of the child being born with serious physical or mental abnormalities. This may occur. (A) If the pregnant woman in the first three months suffers from : (1) German measles, (incidence of congenital defects 10 to 12%). (2) Smallpox or chicken pox. (3) Toxoplasmosis. (4) Viral hepatitis. (5) Any severe viral infection. (B) If the pregnant woman is treated with drugs like thalidomide, cortisone, aminopterin, antimitotic drugs, or if she consumes hallucinogens or antidepressants. (C) Mother is treated by X-rays or radio-isotopes. (D)

Insanity of the parents. (3) **Humanitarian :** When pregnancy has been caused by rape. (4) **Social**: (A) When pregnancy has resulted from the failure of contraceptive methods in case of a married woman, which is likely to cause serious injury to her mental health. (B) When social or economic environment, actual or reasonably expected can injure the mother's health.

Rules: (1) Only a qualified registered medical practitioner possessing prescribed experience can terminate pregnancy. Chief Medical Officer of the district is empowered to certify that a doctor has the necessary training to do abortions. A medical practitioner can qualify if he has assisted in performance of twenty-five cases of M.T.P. in a recognised hospital. (2) The pregnancy should be terminated in Government hospitals, or in the hospitals recognised by the Government for this purpose. (3) Non-governmental institutions may take up abortion if they obtain a licence from Chief Medical Officer of the district. (4) The consent of the woman is required before conducting abortion; written consent of the guardian is required if the woman is a minor or a mentally ill person. Consent of husband is not necessary. (5) Abortion cannot be performed on the request of the husband, if the woman herself is not willing. (6) The woman need not produce proof of her age. The statement of the woman that she is over eighteen years of age is accepted. (7) It is enough for the woman to state that she was raped, and it is not necessary that a complaint was lodged with the police. (8) Professional secrecy has to be maintained. The Admission Register for the termination of pregnancies is secret document, and the information contained therein should not be disclosed to any person. (9) If the period of pregnancy is below 12 weeks, it can be terminated on the opinion of a single doctor. (10) If the period of pregnancy is between 12 and 20 weeks, two doctors must agree that there is an indication. Once the opinion is formed, the termination can be done by any one doctor. (11) In an emergency, pregnancy can be terminated by a single doctor, even without required training (even after twenty weeks), without consulting a second doctor, in a private hospital which is not recognised. (12) The termination of pregnancy by a person who is not registered medical practitioner (person concerned), or in an unrecognised hospital (the administrative head) shall be punished with rigorous imprisonment for a term which shall not be less than two years, but which may extend to seven years. Where a doctor acts under the provisions of the act, various sections of the I.P.C. referring to abortion will not apply to him. The doctor is protected from any legal action for any damage caused or likely to be caused in terminating the pregnancy, provided he has acted in good faith and exercised proper care and skill. Though the Act does not state that it is "abortion on demand", the provisions are extremely liberal for any woman to obtain termination of pregnancy.

THE METHODS OF PROCURING CRIMINAL ABORTION

The methods commonly used for terminating an unwanted pregnancy, can be roughly divided into three periods : (1) Up to the end of the first month, the woman may take violent exercises, hot baths and purgatives. Extreme violence may lead to internal injury. (2) Up to the end of the second month, when suspicion becomes certainty, abortifacient drugs are used. (3) About the third or fourth month, after failing to procure abortion by the above methods, mechanical interference is done either by the woman herself or by some other person.

(1) Abortifacient Drugs ; Every common drug has been used at some time in an attempt to produce criminal abortion. They either produce congestion of the uterine mucosa and then uterine bleeding, followed by contraction of the uterine muscle and expulsion of the foetus, or they cause the uterine contraction by stimulating the myometrium directly. There is no drug which when taken by the mouth causes abortion without endangering the life of the woman.

(1) Drugs Acting Directly on the Uterus : (A) **Ecbolics** : They increase uterine contraction but do not relax or dilate the cervical canal and external os, which is necessary to expel the foetus. Ergot is most commonly used and has a uterine action which increases as pregnancy advances, but its toxic circulatory side-effects result in arterial spasm and gangrene of the extremities. It frequently fails during the earlier months of pregnancy. Hydrastis canadensis has an action similar to but less intense than ergot. Quinine has a direct action upon the uterus or uterine nerves, but its action is not certain. Lead in the form of pills made from diachylon (lead oleate), or lead plaster is commonly used. It causes tonic contractions of the uterus and also has a direct toxic

effect on the cells of the developing ovum. Death of the foetus may occur in doses which do not seriously affect the mother. Symptoms of lead poisoning may occur before abortion takes place. Pituitary extract has a specific oxytocic effect on uterine muscle. Its effect is significant only near the term. Synthetic oestrogens do not have any abortifacient effect except perhaps in very large doses. Decoctions of cotton root bark, nitrobenzol, picrotoxin and strychnine are also used.

(B) Emmenagogues : They produce or increase the menstrual flow. They act as abortifacient when given in large and repeated doses. The chief of these are savin, borax, apiol, rue, laburnum, oestrogens, sanguinarin, senecio, caulophyllin, hellebore, etc.

(2) Irritants of the Genito-urinary Tract : They produce reflex uterine contractions, e.g., oil of pennyroyal, oil of tansy, oil of turpentine, cantharides, etc. They may produce severe inflammation of the kidney in large doses. Potassium permanganate is applied to the vaginal vault in 120 to 300 mg. tablets, crystals or solution. It produces acute localised punched-out ulceration with raised edges and a granular black base and causes severe haemorrhage due to erosion of small arterial vessels.

(3) Irritants of the Gastrointestinal Tract: Any substance which causes irritation of the colon may produce hyperaemia and contractions of uterus. Saline cathartics, such as magnesium sulphate or drastic purgatives, such as aloes, calomel, castor oil, croton oil, jalap, colocynth, phenolphthalein, rhubarb, senna, scammony, podophyllum, elaterium or gamboge are commonly used. The commonly used emetic is tartar emetic.

(4) Drugs having Poisonous Effects on the Body: (A) Inorganic irritants, e.g., lead, copper, iron, mercury and antimony. (B) Organic irritants, e.g. cantharides, unripe fruit of *papaya*, unripe fruit of pineapple, seeds of carrot, *moringa*, etc., juice of calotropis, bark of plumbago rosea, caryophyllus, *methi*, saffron, etc.

(II) General Violence : It acts directly on the uterus, or indirectly by producing congestion of pelvic organs, or haemorrhages between uterus and membranes. It may be successful in those in whom there is some natural irritabiliy of the uterus, but in others who are not naturally predisposed to abort, even violence of a severe degree fails to produce abortion.

(A) Intentional : (1) Severe pressure on the abdomen by kneading, blows, kicks, jumping, tight lacing, etc. and massage of the uterus through the abdominal wall.

(2) Violent exercise, e.g., horse riding, cycling, jumping from a height, severe jolting as driving over a rough road, running upstairs and downstairs and carrying or lifting heavy weights.

(3) Cupping : A mug is turned mouth downwards over a lighted wick and placed on the hypogastrium and the mug is pulled, which results in partial separation of the placenta. This is usually practised in advanced pregnancy.

(4) Very hot and cold hip baths alternately.

(B) Accidental : A general shake-up in advanced pregnancy can produce abortion, but if the ovum is healthy, abortion will not occur.

(III) Local Violence : The choice of the method and its results will depend upon the skill of the operator.

(1) Syringing : The ordinary enema syringe with a hand-bulb is commonly used to inject fluid into the uterus, the hard nozzle being inserted into the cervix. Sometimes, Higginson's syringe is used. The suction valve is placed in a bowl of fluid and pressure applied on the bulb. Due to imperfect filling of the bulb, a mixture of air and fluid is forced into the uterine cavity at a pressure higher than that present in uterine veins. The fluid detaches part of the amniotic sac and placenta from the uterine walls, followed by haemorrhage, uterine contraction and abortion. Soap water is often used as an injection material. Irritating substances may be added to the water, such as lysol, cresol, corrosive sublimate, alum, inorganic acids, potassium permanganate, formalin, turpentine, arsenic compounds, lead compounds, etc. These substances may be absorbed through the vaginal and uterine mucosa and cause toxaemia, shock and death. Extensive tissue destruction may lead to infection and fatal haemorrhage. Death may result from air embolism. The risk of air embolism increases as pregnancy advances and emboli may enter the brain through the placental venous plexus or the abdominal or thoracic veins which anastomose with the vertebral venous plexus. Rough insertion of the syringe into the cervix, or rapid injection of cold or unduly hot fluid, may cause sudden death from vagal inhibition. Douches of hot or cold water may be applied to the vagina or water may be projected with considerable force towards the uterine os. The

injection of fluid can be self-administered or carried out by an abortionist.

(2) Rupturing of the Membranes : The membranes are ruptured by introduction of an instrument, e.g., a uterine sound, catheter, probe, stick, pencil, penholder, umbrella rib, knitting needle, crochet needle, curtain rod, nail, hairpin, piece of wire, glass rod, screwdriver, douche cannula, etc. into the cavity of the uterus. Abortion usually occurs from few hours to two to three days, due to escape of liquor amnii but occasionally may not occur for days or weeks. This can be done by the woman herself or by an abortionist. Instruments or parts of the instruments can break after introduction into the uterine cavity or perforation of vaginal or uterine wall.

(3) Syringe aspiration: A large syringe with a plastic cannula pushed through the cervix, develops suction which ruptures the early gestational sac and leads to aspiration or later expulsion of the contents.

(4) Dilation of the Cervix : Foreign bodies left in the cervical canal, such as pessaries, laminaria tent (a dried seaweed) or seatangle tent, or obturator, dilate the cervix, irritate the uterine mucosa and produce marked congestion and uterine contractions, with expulsion of the foetus. They are used after 8 weeks of pregnancy in nuliparous women and in cases with rigid cervix. The cervical canal may be dilated by introducing a compressed sponge into the cervix and leaving it there. The sponge swells from moisture in the uterine segment with expulsion of the foetus. **Slippery elm bark** is obtained from a tree grown in Central and North America. It occurs in soft flat pieces of varying length and width and about three mm. in thickness. The pieces are cut to the desired length and breadth and inserted into the cervical canal. They absorb moisture and within a few minutes, a jelly-like layer is produced on each side of the bark, which is as thick as the bark itself, due to which cervical canal is dilated.

(5) Abortion Stick : This is a thin wood or bamboo stick, from 12 to 18 cm. long. This stick is wrapped round at one end or for the greater portion of its length with cotton-wool or a piece of cloth, and soaked with juice of marking nut, calotropis, jequirity, asafoetida or paste made of arsenious oxide, arsenic sulphate, mercuric chloride and red lead, etc. It is introduced into the vagina or os of uterus by professional abortionists *(dhais)* and retained there till uterine contractions begin. Instead of this stick, a twig of some irritant plant, e.g., calotropis, nerium odorum, cerbera thevetia, plumbago rosea or zeylanica, etc. is used. In some cases irritating juice is directly applied to the os, or a piece of cloth saturated with irritating juice, or paste is introduced into the vagina.

(6) Air Insufflation : Air is introduced into vagina and uterus by various means, e.g., pumps, syringes, douche tips, and oral-genital contact.

(7) Electricity : The negative pole is placed over the cervix in the posterior vaginal vault, and positive over the sacrum or lumbar vertebrae. When current is passed, uterus contracts and may expel its contents.

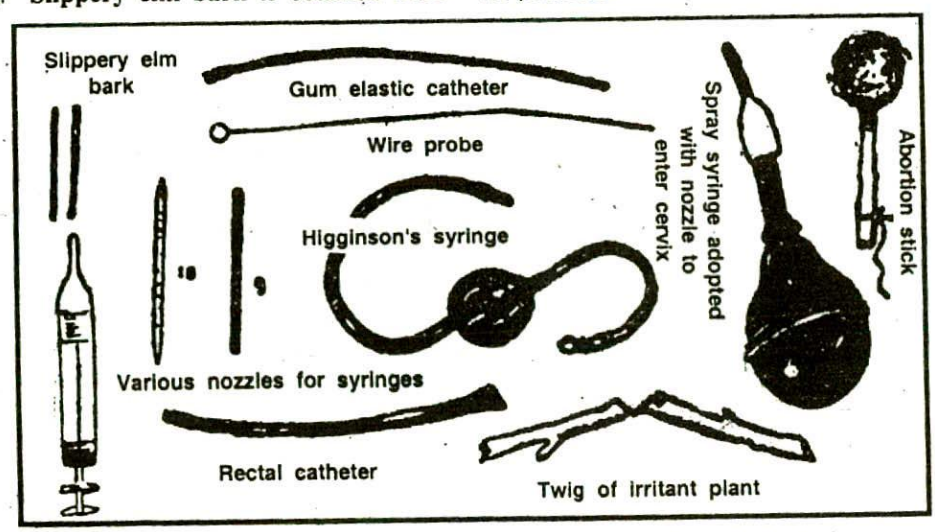

Fig. (18–1) Common instruments used to procure criminal abortion.

(8) Curettage: Some criminal abortions are produced by dilation and curettage under general anaesthesia.

(9) Pastes : Pastes containing iodine and thymol, potassium iodide, or mercury (Utus, Foetex, Indulabo, Interruptin pastes) are injected from a collapsible tube with a uterine applicator. Ten to thirty ml. is injected into the uterus through the external os. The principle is to detach parts of placenta from the uterine wall, similar to the syringing method. They produce necrosis and infection of the uterine wall similar to that produced when soap water is injected into the uterus in a large quantity.

Therapeutic Methods : The common methods are : (1) Low rupture of the membranes.

(2) Utus paste is injected through a cannula into the cervical canal.

(3) Dilatation of the cervix and oxytocic infusion, or a direct injection of ten units of oxytocin into the uterus.

(4) Dilation of the cervix and evacuation of the uterus by curettage, during the first three months.

(5) Prostaglandins : Prostin E_2 (PGE_2), and prostin F_2 (PGF_2) induce labour and abortion. They can be given intravenously, orally or by intravaginal application.

(6) Amniotic Fluid Replacement Therapy: This method is useful after twelfth week of pregnancy, or in cases of intrauterine death. With long needle, amniotic fluid is removed and replaced with equal volume of 20% saline or 50% glucose. Abortion usually occurs in 24 to 48 hours after injection.

(7) Electrical Vacuum Aspiration : This is done during the first three months of pregnancy. The cervix is dilated and the cannula is introduced into the uterine cavity. Between 8 to 12 weeks of pregnancy, a nine mm. cannula is sufficient. A negative pressure of 0.4 to 0.6 kg/sq.cm. is produced in the uterine cavity by means of a vacuum pump for evacuation of the contents. The cannula is moved gently up and down over all sides of the uterine cavity. The contents are broken up by aspiration, and are collected in a bottle connected to the cannula. Aspiration usually takes 3 to 5 minutes. Ease of performance, less haemorrhage and diminished danger of perforation of uterus are the advantages of this method.

(8) Manual Vacuum Aspiration: A hand-held vacuum syringe and flexible plastic cannula are used. The products are aspirated by suction into the syringe. Sedation or anaesthesia are not required

(9) Abdominal Hysterotomy: This method is preferred after 14 weeks of pregnancy.

Abortion and the Medical Practitioner: Sometimes, an abortionist produces an incomplete abortion and sends the woman to the doctor as a case of genuine abortion. A woman may insert a tablet of potassium permanganate into the vagina which causes acute localised ulceration with profuse bleeding due to erosion of small arterial vessels. The patient then visits a doctor who may be led to believe that she is threatened with abortion and may evacuate the uterus. Sometimes, a woman may go to a doctor with the complaint of displacement of the uterus, and the unsuspecting doctor may pass a sound, which may result in abortion.

EVIDENCE OF ABORTION

Criminal abortion should be suspected when: (1) the deceased is pregnant and deeply cyanosed, (2) instruments to procure an abortion or abortifacient drugs are found at the scene of death, (3) the underclothing appears to be disturbed after death, (4) fluid, soapy or blood-stained is coming out of the vagina.

The evidence of criminal abortion is obtained from: (1) The victim's or deceased's medical history and her whereabouts prior to death. (2) The clinical or autopsy examination. (3) The examination of aborted material if available.

IN THE LIVING: In the living, abortion cases come to the doctor: (1) when a woman alleges abortion after a blow or quarrel in order to inflict severe penalty on the accused, (2) when a woman is charged with abortion but wishes to conceal it.

The signs of recent abortion are essentially those of recent delivery, but they depend on the length of pregnancy. The signs are modified depending on the time that has passed between abortion and examination.

In abortion during the first two to three months of pregnancy, the signs are ill-defined and consist of haemorrhage, slight softening of external os and vaginal walls, and slight enlargement of the uterus, which disappear in few days. The breast changes of pregnancy in the case of primipara are useful. During the fourth and fifth months of pregnancy, the haemorrhage is more marked, and the internal os may admit a finger. The os is not injured in abortions of less than six months of pregnancy. The genital organs are much softened and tags of membrane may be found in the uterus. The general condition of the genital tract and the injuries can be made out by visual examination with a speculum. The vaginal canal may show erosions

or lacerations. Cervix may show marks of valsellum forceps, fissures or lacerations, indicating use of an instrument. Signs of injury to the abdomen or body should be noted. Signs of metritis or secondary peritonitis and also any diseases predisposing to natural abortion should be noted.

The organisms causing uterine sepsis following evacuation are: Cl. welchii, E. coli, Streptococcus pyogenes, Staphylococcus aureus and anaerobic Streptococci. Strong antiseptics produce surface tissue necrosis which encourages bacterial growth. In fatal cases, the sepsis usually involves the endometrium, especially the placental site and pieces of retained products. The myometrium, tubes and adjacent pelvic organs and peritoneum are also infected. Material and liquid from the vagina, uterine cavity and blood should be collected for chemical and bacteriological examination. Urine, and vomit should be preserved for chemical analysis.

POST-MORTEM EVIDENCE OF CRIMINAL ABORTION

The following points should be proved to convict the abortionist: (1) that the dead woman was pregnant, (2) that the accused was responsible for the act which resulted in the interruption of the pregnancy, (3) that the accused acted for the purpose of producing an illegal abortion, and (4) that death occurred as the result of the attempt to interrupt the pregnancy. The findings depend upon the mode of abortion practised and the time which has passed between its performance and death.

The material alleged to have been expelled from the uterus should be examined for products of conception. If recognition is not possible, the material should be placed in water to dissolve the blood. A portion should be examined microscopically to know whether it is a blood clot, foetus, polyp or fibroid. In the case of blood clot, grouping and precipitin test should be done to know whether it is compatible with the woman's blood groups. In the case of a foetus, the age should be determined.

. Evidence at the Scene: The doctor should make a note of: (1) Condition of the bed-linen (blood-stained, soiled, etc.). The arrangement of clothing should be noted, especially the underclothing. The soaked clothes may smell of carbolic soap or dettol. (2) Any signs of recent interference of delivery. (3) Any evidence of discarded linen, dressings, cotton-wool, swabs, bowls, etc. (4) Presence of any known abortifacient drug. The presence of a second person is indicated by the absence of instruments at the scene, if subsequent autopsy reveals that death was due to an instrumental abortion.

(1) Abortion by the Drugs: The gastrointestinal tract must be examined for evidence of irritant poisoning. If such evidence is found, the whole tract and its contents together with other organs of the body should be preserved for analysis. The urinary tract should be examined for signs of inflammation, such as might be produced by cantharides or turpentine. The vagina and cervix should be examined for erosions and inflammation due to local application of irritant and caustic substances, and they should be preserved for analysis.

(2) Instrumental Abortion: In many cases, injuries are present in the vagina, cervix, uterus or its contents including any dilation of the cervix. Gross injury when present indicates lack of skill or knowledge of the parts. In skilled hand, instrumentation may leave little or no evidence of any kind, but infection if present is suspicious of interference, unless the foetus is macerated or products are retained. In natural abortion, infection is rare. The nature of injury may indicate whether the instrument is penetrating and sharp-pointed or blunt-pointed, but the exact nature cannot be made out. In self-practised interference the damage is great, but when done by another person the damage is slight. Rarely, self-instrumentation may not cause any damage.

Signs of injury to the abdomen or body may or may not be present. The abdomen is opened, and before removing any parts from the cavity, the peritoneum, pelvic organs and floor are examined for punctures, ruptures, haemorrhage or inflammation. If embolism is suspected, the presence of air in the large veins and in the heart should be looked for, before the organs are removed. The genital organs

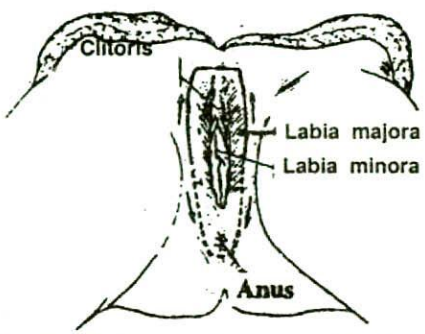

Fig. (18–2) Incision for removal of the female external genitalia.

are removed *en masse* by making an incision just outside the labia majora and extending it backwards to include the anus, and forwards up to the symphysis pubis, which is then divided. The peritoneum is cut along the brim of the pelvis and all pelvic viscera, including uterus, vagina, anus, rectum and bladder are removed. The organs are examined externally and then opened, adopting the incisions so as to avoid an injured part. The uterus is opened first by a classical caesarean incision. This will facilitate the collection of any fluid retained in the lower uterine segment. Then the uterus is opened in its long axis and the colour, size and texture noted. If the chorionic sac is still present, its integrity and the attachment to deciduae are noted. Products of conception are sought and the state of the placental bed noted. Next, the cervical canal is opened and examined for injuries. The vaginal canal, and especially the posterior fornix should be examined for signs of lacerations, bruising, perforations and inflammation and foreign material. Note state of dilatation of cervical canal. Wounds of the cervix occur in about fifty percent of the cases, which may be groove-like, parallel notches of the cervical canal, tenaculum marks on the external orifices, cavitation of the wall, fissures, lacerations and perforations. The perforations of the vagina and uterus are of different sizes and forms varying from a small ragged, stellate opening about one cm. in diameter to much larger tears of stellate, oval or irregular shape. Sometimes, one or more perforations may be present in the fundus. Ragged, stellate or irregular tears of small or large intestine, mesentery, omentum and bladder may occur. Caecum, sigmoid and rectum are usually involved. Segments of the intestine, omentum or mesentery may enter the uterine cavity. Sometimes, foreign bodies may be found in the genital tract which should be preserved. Material and fluid from the vagina, uterine cavity, and blood from both ventricles should be collected for chemical and bacteriological examination. Tissue from the uterus, ovaries and other organs must be taken for histological examination. This is especially important in cases where there is no evidence of foetal parts or placental remains. Bacteriological examination should also be done on possible instruments of abortion. Fluid from the cut surface of the lung and pulmonary blood should be collected for fatty acid estimation and for phenolic derivatives depending on the agent used. A full photographic record should be maintained.

(3) Abortion by Syringing: There may be fluid in the vagina and soiling of clothes. The mucous plug in the cervix is usually displaced or disintegrated. Corrosion or tissue damage may be seen due to the use of antiseptics. The cervical canal may be dilated and injured. Foamy-red or dark-red fluid may be seen between the uterine wall and foetal membranes with partial detachment of the placenta. The injected fluid enters the uterine sinusoids under pressure and the fluid and bubbles of gas can be detected in the venous system extending from the sides of the uterus up to right heart. The right side of the heart, the superior and inferior vena cavae, and pulmonary conus contain foamy blood and are "ballooned out" and have a characteristic elastic feel. When fatal venous air embolism has occurred, the inferior vena cava, uterine, ovarian and pelvic veins present a beaded appearance due to the air within their lumens. The large abdominal veins should be examined for the presence of air by gently moving aside the bowel before the thoracic cavity is opened and the internal mammary vessels are incised. Segmentation of the coronary vessels is seen due to gas bubbles. In air embolism, collapse occurs in about two minutes and death in ten minutes. Delayed death may occur when the victim is at rest and the air is temporarily locked in the uterus. When the woman moves about, utero-placental detachment

Fig. (18–3). Criminal abortion (five months pregnancy). The severed foetal head is retained in the uterus.

increases and air enters uterine sinusoids. Perforation may occasionally result. In self-induced cases, the woman may be found dead in a posture consistent with recent use of a syringe. Usually the apparatus is by her side, but she may have had time to dispose it off.

FABRICATED ABORTION: Rarely, when a woman is assaulted, she may try to exaggerate the offence by alleging that it caused her to abort. She may acquire a human foetus, or an animal foetus to support the charge.

CASE : A woman claimed that she had been assaulted while pregnant, and as the result of violence had aborted. The products of the alleged abortion were sent, which on examination was found to be 'foetus' of a dog. The forelegs and tail had been clipped off and the remains bottled.

Doctor's Duties in a Case of Criminal Abortion: (1) The doctor should keep all the information obtained by him as a professional secret. (2) He must ask the patient to make a statement about the induction of criminal abortion. If she refuses to make a statement, he should not pursue the matter. (3) He must consult a professional colleague. (4) He must treat her to the best of his ability. (5) If the woman's condition is serious, he must arrange to record the dying declaration. (6) If the woman dies, he should not issue a death certificate, but he should inform the police.

MEDICO-LEGAL IMPORTANCE OF PLACENTA: (1) It gives an idea of the length of gestation. (2) In criminal abortion, often pieces are retained in the uterus. (3) The transfer of poisons, bacteria, antibodies, etc. across the placenta may result in death, disease or abnormalities of the foetus.

TRAUMA AND ABORTION : Trauma may rarely cause an abortion in the absence of serious or life-threatening injury to the mother. Often it is difficult to establish a causal relationship between trauma and abortion. An abortion occurring within a few days of the trauma may be in progress at the time of injury. Trauma producing embryonic injury may not cause abortion for several weeks or even months. Most of such complications occur without any trauma. Trauma which seriously injures the mother, rarely produces abortion. Travel in the absence of trauma does not increase the incidence of abortion. Because of the above inconsistencies, it is often difficult to answer whether trauma, such as traffic accident, a fall, an assault, etc. can cause abortion. Without any known cause abortion may occur, the foetus may die, or the placenta separate.

Rupture of membranes or premature separation of placenta may occur in some cases, directly due to trauma.

The following criteria suggest a causal relationship between trauma and abortion. (1) The traumatic event was followed within twenty-four hours by a process that ultimately led to abortion. (2) The foetus and placenta should be normal. (3) The appearance of the foetus and placenta should be compatible with the period of pregnancy at which the traumatic event occurred. (4) Factors known to cause abortion should be absent, such as: (a) abnormalities of the uterus including congenital defect of uterine development, leiomyomas, endometrial polyps and incompetent cervical os; (b) a history of repeated abortion without any cause; (c) chronic infections in the mother, e.g., syphilis, or toxoplasmosis involving the uterus; (d) history of exposure to abortifacients, e.g., X-ray, lead, folic acid antagonists; (e) a physical attempt to induce abortion. (5) Adherent clot or a depression of the placental surface.

Table (18–1) Difference between natural and criminal abortion.

	Trait	Natural abortion	Criminal abortion
(1)	Cause:	Predisposing diseases.	Pregnancy in unmarried woman or widow.
(2)	Infection :	Rare.	Frequent.
(3)	Marks of violence :	Not present on the abodmen.	May be present on abdomen.
(4)	Genital organs :	Injuries are not present.	Injuries, such as contusions, lacerations, perforations, etc. may be seen in uterus or its contents and vagina.
(5)	Toxic effect of drugs :	Absent.	Erosions and inflammation of vagina and cervix due to local application of irritant and caustic substances may be present. The G.I. or urinary tract may show signs of irritation.
(6)	Foreign bodies :	Not present in genital tract.	May be present in genital tract.
(7)	Foetus :	Wounds absent.	Rarely wounds may be present.

Trauma may cause foetal injury or death. The age of the injury should correspond with the time passed after trauma. Soft tissue injuries heal in the uterus. In the case of fractures, the process can be dated by X-ray. A relationship may be established, if the age of fracture coincides with the traumatic event. Fractures of the skull and long bones can occur during delivery. To differentiate birth trauma from accidental trauma, X-rays are very helpful.

MEDICO-LEGAL QUESTIONS: The doctor should be able to answer the following questions after autopsy. (1) Was the deceased pregnant recently? (2) Was there any evidence of abortion? (3) Was there any evidence of criminal interference by instruments or the use of drugs? (4) Was the cause of death related to abortion?

INSTRUMENTAL ABORTION : The following questions arise in the case of instrumental abortion.

(1) HAS ANY INSTRUMENT BEEN USED, AND IF SO, WHEN?

The presence of any injuries in the vagina, cervix, uterus or its contents and dilation of the cervix or injection of fluid will indicate the use of an instrument. If the abortionist is skilled, the injuries are likely to be minimal. The age of the injuries should be determined by microscopic examination. Gross injuries indicate lack of skill or knowledge of the parts. Recent dilation of the cervix may be difficult to differentiate from cervical relaxation during passage of the foetus. The injected fluid may be washed out by the floodings of an abortion. In such case, the contents of the fluid, e.g., soap, phenol, potassium permanganate, etc., can be detected in the blood, obtained from the inferior vena cava and the pulmonary arteries. In the absence of injuries, the development of infection should arouse suspicion of interference, unless the foetus is macerated or products are retained.

EVIDENCE ON THE TIME OF INTERFERENCE: The timing of the passing of an instrument can be better judged from the course of complications rather than of abortion itself. Vagal inhibition will produce instantaneous death. Death from air embolism occurs within seconds or a minute but delayed collapse and death may occur, probably due to delay in separation of the placenta. In such cases, an abortion may have been carried out in the house of the abortionist, but the patient may reach her own home before she collapses and dies. In haemorrhage, death occurs after some hours, depending on the amount of bleeding. Clostridial infection may develop within 24 hours and due to other bacteria in a day or two. Mixed infections associated with phlebitis or pyaemia may last for weeks. If placental remains are responsible, death may occur after several days, and if due to sepsis after weeks or months. When abortifacient drugs have been used, the timing of administration can be estimated from the development of toxic signs or symptoms. Due to the unreliability of the witnesses, in most cases it is not possible to estimate the interval between instrumentation and death.

(2) WHAT KIND OF INSTRUMENT WAS USED ? The nature of the injuries may indicate whether the instrument is penetrating or non-penetrating and sharp-pointed, blunt-pointed, hard-nosed, or soft-nosed, but the exact nature cannot be made out. Injecting instruments usually do not produce injuries. Marks produced by surgical forceps, tenaculum, etc., during treatment may cause confusion. The doctor who attended on the patient should be shown the specimen to exclude any marks made by him.

(3) HOW MAY ANY INJURIES PRESENT BE INTERPRETED?

The doctor should determine the type of instrumental interference, i.e., dilating, perforating, injecting, etc., from the available facts. Necrosis and infection may supervene in areas of bruising and internal laceration of the cervix or uterus, which may cause difficulty in interpretation.

(4) WAS INSTRUMENTATION SELF-INDUCED OR ASSISTED?

Gross damage indicates self-instrumentation and minor injuries or absence of injuries indicate skilled assistance.

(5) HOW DID DEATH OCCUR?

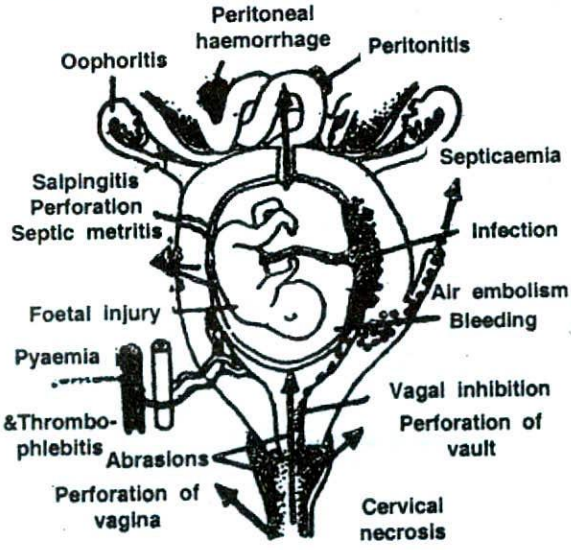

Fig (18–4) Complications of criminal abortion.

The causes of death could be:

(A) **Immediate Deaths**:(1) Vagal inhibition. (2) Air embolism. (3) Haemorrhage. (4) Fat embolism. (5) Amniotic fluid embolism. (6) Rarely poisoning.

(B) **Delayed Deaths** (onset within 48 to 72 hours): (1) Septicaemia. (2) Pyaemia. (3) Confined local infection and toxaemia. (4) General peritonitis. (5) Tetanus.

(C) **Remote Deaths:** (1) Jaundice and renal failure. (2) Bacterial endocarditis. (3) Pulmonary embolism.

AMNIOTIC FLUID EMBOLISM: Most cases of amniotic fluid emboli occur during active labour. Pelvic trauma of parturition, including rupture of uterus, instrumental interference in late pregnancy may allow opening of the sinuses in the placental bed with consequent escape of amniotic fluid. It can also occur in first and second trimester abortions, and following abdominal trauma and amniocentesis. The amniotic fluid enters into the maternal venous circulation with resultant pulmonary microvascular obstruction. The solid elements are rarely found in the liver, kidney and brain. On reaching the lung, this material is presumed to produce severe transient vasospasm of the pulmonary vasculature, pulmonary hypertension, right heart failure, and hypoxia. The exact mechanism of death is now known. In fifty percent of cases death occurs in the first hour. If death does not occur immediately, it causes disseminated intravascular coagulopathy, and fibrin deposition in many organs. In addition to the solids, the actual fluid itself may cause the allergic response that may cause severe collapse and death. The diagnosis is histological and depends upon the demonstration of mucin, foetal squamous cells, meconium, lanugo hair, vernix, fat globules, cells from chorion and amnion in the pulmonary vasculature in sections of the lung stained with H & E. Laboratory studies show decreased fibrinogen, elevated levels of fibrin split products, prolonged partial thromboplastin and prothrombin times, and thrombocytopaenia.Immunohistochemical techniques have also been used in the lung sections to demonstrate human keratin, amniotic fluid derived mucin and isolated trophoblastic cells.

CHAPTER 19
INFANT DEATHS

According to the Infanticide Act of England (1938), **infanticide** means the unlawful destruction of a child under the age of one year. Only the mother can be charged with the offence when the circumstances justify it, such as when the infant is killed by its mother while suffering from disease of the mind due to the effect of stress associated with her pregnancy, delivery, puerperium or lactation. In such cases, the mother may not be held wholly responsible for killing the infant. In India, there is no such special Act, and as such there is no distinction between the murder of newborn infant and that of any other individual. **Foeticide** is the killing of the foetus at any time prior to birth. **Filicide** is the killing of a child by its parents. **Neonaticide** is the deliberate killing of a child within 24 hours of its birth. Infanticide does not include the death of foetus during labour, when it is destroyed by craniotomy or decapitation. Infanticide is rare and usually committed by a young unmarried woman or widow. Infanticide is usually committed at the time of, or within a few minutes or hours after the birth. The alleged mother should be examined for signs of recent delivery and her mental condition should be noted. In case of the child, the points to be decided are: (1) Whether the child was stillborn or deadborn? (2) Whether the infant has attained viability or not? (3) Whether the child was born alive? (4) If born alive, how long did the child live? (5) What was the cause of death?

STILLBIRTH : A stillborn child is one, which is born after twentyeighth week (reduced to 24 weeks in U.K.) of pregnancy, and which did not breathe or show any other signs of life, at any time after being completely born. The child was alive in utero, but dies during the process of birth. Stillbirths occur more frequently among illegitimate and immature male children in primiparae. The incidence is about five percent. It is born in sterile condition, and as such, putrefaction occurs from without inwards, whereas in case of newborn child which lived for some time, the bacteria inside the body may cause putrefaction to start in the abdomen. Signs of prolonged labour, i.e., oedema and bleeding into the scalp, a caput succedaneum, and severe moulding of the head indicate stillbirth or death from natural causes shortly after birth.

Common causes of stillbirth are: prematurity, anoxia of various types, birth trauma especially intracranial haemorrhage due to excessive moulding, placental abnormalities, toxaemias of pregnancy, erythroblastosis foetalis, and many types of congenital defects.

DEADBIRTH : A deadborn child is one which has died in utero, and shows one of the following signs after it is completely born.

(1) Rigor mortis at delivery.

(2) Maceration : Maceration is a process of aseptic autolysis, and is the usual change. This occurs when the dead child remains in the uterus for about three or four days surrounded with liquor amnii but the exclusion of air. Signs of maceration are not seen, if the child is born within 24 hours after death. If air enters the liquor amnii after death of the foetus, putrefaction occurs instead of maceration.

The earliest sign of maceration is skin slippage, which can be seen in 12 hours after the death of the child in utero. Gas in great vessels (aorta in 12 hours) of foetus indicates foetal death. Collapse of the vertebral column occurs. The body of a macerated foetus is soft, flaccid and flattens out when placed on a level surface. It has a sweetish, disagreeable odour. The skin is red or purple. Large blebs appear at 24 hours, which contain a red serous or serosanguineous fluid. The epidermis detaches easily and leaves moist and greasy areas. The tissues are reddish due to haemolysis and oedematous. The abdomen is distended. The serous cavities may contain a turbid reddish fluid. The bones are flexible and readily detached from the soft parts. The joints become abnormally mobile. The skull bones are separated and the brain has a greyish-red pulpy appearance. All the viscera become soft and oedematous and lose their morphology, but lungs and uterus remain unchanged for a long time. The umbilical cord is red, smooth, thickened and soft.

SPALDING'S SIGN: Loss of alignment and overriding of the bones of the cranial vault occur due to shrinkage of the cerebrum after death of the foetus. In the early stage, there is only loss of alignment without overriding. The sign will develop

earlier with a vertex presentation than with a breech. It may be detected within a few days of death of the foetus, but often takes much longer time, sometimes even two to three weeks.

(3) **Mummification:** Mummification occurs when the foetus dies from deficient supply of blood, when liquor amnii is scanty, and when no air enters uterus.

VIABILITY OF THE INFANT: Viability means the physical ability of a foetus to lead a separate existence after birth apart from its mother, by virtue of a certain degree of development. A child is viable after 210 days of intrauterine life, and in some cases after 180.

The smallest baby ever to survive weighed 242 gm. and measured 25 cm.

Livebirth: It means that the child showed signs of life when only part of the child was out of mother, though the child may not have breathed or completely born. The causing of death of such a child is regarded as homicide.

SIGNS OF LIVEBIRTH

In civil cases, any sign of life after complete birth of the child is accepted as proof of livebirth, e.g., hearing a cry, seeing movement of the body or limbs, muscle contractions, etc. The muscles may twitch for some time after death, and therefore it is not safe to assume that twitching of muscles indicate life. A child may cry either in the uterus or in the vagina, which may be heard by bystanders or even outside the room of delivery. This occurs only when the membranes have ruptured and air has entered the uterus. The law presumes that every newborn child found dead was born dead until the contrary is proved. In criminal cases, signs of livebirth have to be demonstrated by post-mortem examination of the child. Internal examination may provide strong, but not definite evidence of a livebirth.

(I) Shape of the Chest : Before respiration, the chest is flat and its circumference is one to two cm. less than the abdomen at the level of the umbilicus. After respiration, the chest expands and becomes arched or drum-shaped.

(II) The Position of the Diaphragm : The abdomen should be opened before the thorax, and the highest point of the diaphragm is noted, which is found about the level of fourth or fifth rib if respiration has not taken place, and at the level of the sixth or seventh rib after breathing. The position is affected by gases of decomposition.

(III) Lungs : Breathing causes important and permanent changes in the lungs, the extent of which depends on the physical strength and period of respiration.

(1) Volume: Unrespired lungs appear smaller, being collapsed on to the hilum when the thorax is opened. Fully respired lungs fill the pleural cavities, and the medial edges overlap the mediastinum and part of the pericardium.

(2) Margins: Before respiration the margins are sharp, which become rounded even when the breathing is feeble. Glistening bullae appear along the margins when there has been a struggle to breathe due to some mechanical obstruction.

(3) Consistency: Before respiration, the lungs are dense, firm, and non-crepitant like liver. After respiration they are soft, spongy, elastic and crepitant. The lungs are also crepitant in putrefaction and after artificial inflation.

(4) Colour and Expansion of the Air Vesicles: Before respiration, lungs are uniformly reddish-brown, bluish or deep-violet, according to the degree of anoxia. The surface of the lobules is marked with shallow furrows. On section, interior of the lung is uniform in colour and texture, and little frothless blood exudes on pressure. After respiration, the air cells become distended with air, usually about

Table (19–1). Difference in lungs before and after respiration

	Trait	Before respiration	After respiration
(1)	Weight:	1/70 of body weight.	1/35 of body weight.
(2)	Volume:	Normal or small.	Larger, and cover the heart.
(3)	Consistency:	Dense, firm, non-crepitant.	Soft, spongy, elastic, crepitant.
(4)	Margins:	Sharp.	Rounded.
(5)	Colour:	Uniformly reddish-brown or bluish-red.	Mottled or marbled appearance.
(6)	Air vesicles:	Not inflated.	Inflated.
(7)	Section:	Little frothless blood exudes on pressure.	Abundant frothy blood exudes on section.
(8)	Floatation :	Whole and parts sink in water	Expanded areas or whole float in water.

the anterior surfaces and margins, and then on the remaining portions of the lungs. As the air vesicles expand, they become raised slightly above the surface, and may be seen as polygonal or angular areas on the surface of the lung, giving it a fine mosaic appearance. As the blood becomes aerated in the expanded area, the colour becomes a light-red, or pink, and the whole lung has a mottled or marbled appearance, with rose-coloured patches of expansion and aeration alternating with the collapsed dark bluish-red areas. If there is complete expansion of the lungs, they usually float and support the heart on the surface of the water. On section, frothy blood exudes from the cut surfaces on slight pressure. Exposure to air will brighten the colour of the foetal lungs, but the air cells are not distended. Mottling is absent in artificially inflated lungs, and on section the exposed surface will exude little blood but no froth.

(5) Gas: In putrefaction, bubbles of gas are seen under the pleura which can be moved from place to place by stroking with the finger. Interstitial blebbing indicates decomposition.

(6) Blood in the Lung Beds : The amount of blood in the lungs after respiration is about twice that in circulation in the stillborn.

(7) Weight : (a) **Static Test or Fodere's Test:** The lungs are ligated across their hila and separated. The average weight of both lungs before respiration varies from 30 to 40 g., and after respiration from 60 to 66 g. The increase in weight is due to the increased flow of blood.

(b) Ploucquet's Test: The blood flow in the lung beds is so increased after breathing that their weight is almost doubled from 1/70 of the body weight before respiration to 1/35 after respiration. The increase in weight is not constant and is not a reliable indication of breathing.

(8) The Hydrostatic Test (Raygat's test) : It is based on the fact that on breathing, the volume of the lungs is increased, which more than compensates the weight of the additional blood, due to which their specific gravity is diminished. The specific gravity of the lungs before respiration varies from 1040 to 1050, and after respiration about 940. A ligature is tied on the bronchi, and lungs separated. Each lung individually is placed in water. If they float, each lung is cut into twelve to twenty pieces and placed in water. A small piece of liver may serve as control. If the liver floats, the test is of no value. If these pieces float, they are each squeezed in between thumb and index finger under the surface of water, to see if any bubbles of air escape, and if they still persist to float, or they are taken out of water, wrapped in a piece of cloth and squeezed by putting a weight to remove the tidal air. The pieces are again placed in water, and if they continue to float, due to the presence of residual air, it indicates that respiration has taken place. If the pieces sink after pressure, respiration has not taken place. If some pieces float while others sink, it shows feeble respiration. A piece of lung is rolled gently between the finger and thumb very near to the ear. A crackling crepitant noise indicates a significant degree of respiratory activity. The hydrostatic test is not of much value, because the lungs of the liveborn who have lived for few days may sink, and the lungs of the stillborn may float.

The expanded lungs may sink from : (1) **Disease,** e.g. acute oedema, pneumonia, congenital syphilis, etc. (2) Atelectasis (non-expansion) of the lungs due to: (1) Air not entering the lungs due to feeble respiration but aeration of lungs may occur through the mucosa of trachea and bronchi. (2) Complete absorption of air from the lungs by the blood, if circulation continued after stoppage of respiration. (3) More air being expelled from the lungs during expiration than what is inhaled during inspiration, if the respiratory movements are very feeble. (4) Obstruction by an alveolar duct membrane.

The unexpanded lungs may float from: (1) Putrefactive gases : The putrefied lungs are soft and greenish. Bubbles of gas may be seen on surface of the lung, which are large and not uniform, project considerably from the surface, and the gas in them can be pushed readily from place to place, and bubbles collapse on pricking. Signs of decomposition of the body will be seen. (2) **Artificial inflation :** The foetal lungs may be artificially inflated by blowing air through a tube, catheter or cannula passed into the trachea or by the mouth-to-mouth method. In such cases, the lungs can be inflated only partially and the stomach contains air.

Hydrostaic test is not necessary when : (1) The foetus is a monster. (2) The foetus is macerated or mummified. (3) The foetus is born before 180 days of gestation.(4) The stomach contains milk. (5) The umbilical cord has separated and a scar has formed.

Signs of the struggle to breathe: The victims

of the "struggle to breathe" may be stillborn or they die shortly after delivery. The gross changes in both the groups are similar. The sequence of changes, as the struggle to breathe develops is as follows: (1) Dark fluid blood (due to raised CO_2), haemoconcentration, "pseudo-clots". (2) Cyanosed, expanded lungs. Expansion due to (a) inhaled liquor amnii or vernix, (b) obstructive emphysema, (c) oedema with or without air or liquor amnii. (3) Tardieu's spots on pericardium, pleura and thymus. (4) Liver swelling due to congestion of whole lobule. (5) Distension of large bowel with meconium. (6) Ascites. (7) Retroperitoneal oedema.

Respiration Before and During Birth : A child may breathe: (1) while it is in the womb, after the rupture of the membranes **(vagitus uterinus)**, (2) while its head is in the vagina **(vagitus vaginalis)**, (3) while its head is protruding from the outlet. A child which has breathed in the uterus or vagina may die from natural causes, before it is completely born. Therefore, proof of breathing is not proof of livebirth. In the newborn child, the respirations may not be strong or deep enough to expand the air cells, and the child may live for some time on oxygen absorbed from the respiratory cells of the alveolar ducts. Some air may pass into the cells, but may not be sufficient to distend the fibrous tissue. This air may be subsequently absorbed by the blood, or may be lost. The child may live for many hours or even one or two days with only small portion of its lung tissue expanded. When the air cells have been distended once, they never return to the foetal condition. For the above reasons, the hydrostatic test fails in a small percentage of cases.

Microscopic Examination of the Lungs : Microscopic examination is of value in determining the extent of respiration, and the presence of pulmonary disease or abnormality, which may have caused or contributed to the death. The thoracic contents are removed intact by cuts with a scalpel by "no touch" technique of Osborn (1953), to eliminate artifacts. They are fixed for 48 hours and sections are taken of the whole lung in cross-sections. At four months pregnancy, the parenchyma of the lung has gland-like structure with a cuboidal or columnar cell lining. After fifth month, the air sacs are filled with amniotic fluid. The thin-walled adult type of alveolus is formed before full term. At full term, the normal foetal lung is almost completely atelectatic but many of its terminal bronchioles and vesicles are partly expanded by amniotic fluid. The foetus towards term normally makes respiratory movements which fill the alveoli with amniotic fluid. This material is not stained with haemotoxylin and eosin, giving the impression that alveoli have been well expanded by air. With respiration, the alveoli further expand and the fluid is partly expelled through the bronchi and partly absorbed back into the pulmonary circulation. It was thought that if respiration has not taken place, the alveoli appear as hollow gland-like structures lined by cuboidal or columnar epithelium. This is not correct, for the changes in the type of cell which lines the air sacs does not occur with the onset of respiration. If the child has lived only for a few minutes, microscopy cannot always provide clear evidence of extrauterine respiration. Obstruction of the lower bronchial tree by hyaline duct membrane causes respiratory failure. Its development and clinical features are obscure, but its presence is evidence that death is due to natural cause. The struggle to breathe may result in: (1) incomplete lung expansion, (2) suboxia and cyanosis, (3) petechial haemorrhages mainly subpleural, (4) oedema of the mediastinum and often of the lung.

Microscopic examination of the lungs is not helpful. In a child who has breathed, diffuse atelectasis may be seen, and in a stillborn child open apparently aerated alveoli.

Tests for foetal lung maturity are: lecithin, creatine and fat cells.

Livebirth is probable when: (1) All the lobes of the lungs are fully expanded with or without obstructive emphysema. (2) There is oedema of the lungs, especially gross. (3) An alveolar duct membrane is present and has widespread distribution in the lungs. (4) Pulmonary atelectasis due to obstruction by an alveolar duct membrane is present. (5) Contusions of the lungs are present.

A gland-like appearance of the alveoli does not exclude livebirth but only indicates prematurity. Tardieu's spots may be present both in stillbirth and livebirth and also in bronchopneumonia.

Stillbirth is probable in the presence of: (1) Maceration of the infant. (2) Flooding of the lungs with liquor amnii, and especially evidence of phagocytosis of meconium by the cells lining the air sacs. (3) Desquamation of bronchial epithelium. (4) Distention of large bowel with meconium indicating a struggle to breathe.

(IV) Changes in the Stomach and Intestines: Air is swallowed into the stomach during respiration. The stomach and intestines are removed after tying double ligatures at each end. They float in water

if respiration has taken place, otherwise they sink. This is known as **Breslau's second life test, or stomach-bowel test.** This test is not of much value because air may be swallowed by the child in attempting to free the air-passages of fluid obstructions in cases of stillbirth. It is useless when there is decomposition.

In a stillborn child or one dying shortly after birth, the stomach will contain grey-white gastric mucin mixed with swallowed amniotic fluid. Sometimes, the infant may swallow maternal blood during delivery. When dissected under water, the stomach shows mucus, saliva and air bubbles if respiration has taken place, and only mucus if breathing has not occurred. Blood, meconium or liquor amnii in the stomach indicate that the child was alive at or shortly before birth. If milk is present in the stomach, it is a positive evidence that the child has lived for some time after birth.

(V) Changes in the Middle Ear: (Wredin's Test). Before birth, the middle ear contains gelatinous embryonic connective tissue. With respiration, the sphincter at the pharyngeal end of eustachian tube relaxes and air replaces the gelatinous substances in few hours to five weeks. This is not at all reliable.

(VI) Other Signs of Livebirth: Several changes occur in the child after birth, which are helpful in estimating the length of time the child lived after birth.

(1) Blood: Nucleated red cells usually disappear from the blood within 24 hours. Foetal haemoglobin (synthesised mainly in liver) which is about 80% to 90% before birth rapidly decreases to 7 to 8 percent at third month.

(2) Meconium: It is the green viscid substance consisting of thickened bile and mucus. The meconium is completely excreted from the large intestine in the first 24 to 48 hours after birth, but in a breech presentation and also in severe anoxia, the meconium may be excreted completely before birth. Meconium stains are brownish-green and stiffen the cloth. The reaction is acid.

(3) Caput Succedaneum : This is an area of soft swelling that forms in the scalp over the presenting part of the head in vertex presentations. The elevated rounded area of the caput succedaneum corresponds to the portion of the scalp surface that is exposed within the opening of the dilated cervix during labour. The scalp in the area of the caput is swollen to three to four times its normal thickness. The localised area of oedema and congestion is due to local interference with venous return produced by the pressure of the rigid cervical ring. Most commonly, the caput occurs asymmetrically over the crown of the head, in the parietal region. With breech presentations, similar swellings occur over the buttocks and scrotum or labia. The caput succedaneum gradually diminishes often disappearing during the first day (within a week) after birth.

Cephalhaematoma : This is a localised accumulation of blood deep to the scalp, between the periosteum and bone surface. The haematoma is limited to the periosteal sheath of single bone, commonly the right parietal bone, and never crosses a suture line. Cephalhaematoma is rare, occurring in less than one percent of newborns, and varies in size from one to 5 cm. The haematoma swelling often tends to increase during the first day or two after birth, as more and more blood accumulates, but gradually decreases in subsequent weeks as the blood is absorbed. Neonatal jaundice may be increased because of the extra load of blood pigment.

(4) Skin : At first skin is bright red, which becomes darker on second or third day, then brick-red, yellow and normal in about a week. Physiological jaundice is seen by third day due to relative insufficiency of enzymes required for conjugation and excretion of bilirubin. **Vernix caseosa** covers the skin, mostly in the axilla, inguinal region and folds of the neck, buttocks and persists for one or two days. Sometimes it may be absent at birth and it is removed by washing. The skin of the abdomen exfoliates during the first three days after birth.

(5) Air in G.I. Tract : Air moves along the gastrointestinal tract at the same speed in full term

Fig. (19–1). Caput succedaneum.

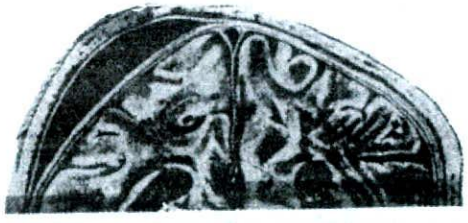

Fig. (19–2). Cephalhaematoma over the right parietal bone.

infants as in premature ones. The air reaches stomach after fifteen minutes; the small intestine after one or 2 hours; the colon after 5 to 6 hours; and the rectum after 12 hours. Bacterial gas formation and resuscitation attempts may be a source of error.

(6) Umbilical Cord : The blood clots in the cut end 2 hours after birth, and the vessels begin to be closed in about 24 hours. The cord attached to the child shrinks and dries in 12 to 24 hours but this appearance is also seen in the body of a stillborn infant , and an inflammatory ring forms at its base in 36 to 48 hours. It mummifies on second or third day. Mummification of the cord also occurs after death if exposed to air. The cord falls off on the fifth or sixth day and leaves an ulcer, which heals and forms a scar in 10 to 12 days.

If a placenta is found with the body, it should be examined. If placenta is absent, the length of the remaining umbilical cord should be measured. Examine the cord to see whether a ligature has been applied and to know whether it has been cut or torn. A torn cord is usually ragged.

(7) Circulation : Contraction of the umbilical arteries starts in about ten hours and are completely closed by third day. The umbilical vein and ductus venous are closed on the fourth day. The ductus arteriosus closes by tenth day, and foramen ovale by second or third month.

CAUSES OF DEATH : Natural Causes : (1) Immaturity. (2) Debility due to lack of general development. (3) Congenital diseases, e.g. syphilis and specific fevers, such as smallpox, plague, etc. affecting the mother, or disease of the child's internal organs, such as lungs, heart, brain, etc. (4) Malformations. (5) Haemorrhage from the umbilical cord, genital organs, stomach, rectum, etc. (6) Post-maturity. (7) Pre-eclamptic toxaemia. (8) Disease of the placenta or its accidental separation from the uterine wall. (9) Placenta praevia or abnormal pregnancy. (10) Neonatal infection. (11) Intrapartum or ante-partum anoxia. (12) Cerebral birth trauma. (13) Erythroblastosis.

UNNATURAL CAUSES : These may be: (1) Accidental, and (2) Criminal.

(I) ACCIDENTAL CAUSES : (A) DURING BIRTH: (1) PROLONGED LABOUR :Severe compression of the head against contracted or deformed pelvis may cause intracranial haemorrhage and death with or without fissured fracture of the parietal bones of the skull. Extradural haemorrhage is rare. Subdural haemorrhages are common and usually bilateral. They are usually caused by rupture of the bridging veins, but may also occur less commonly from tears of the falx cerebri. Rarely, haemorrhages occur from ruptures of the internal or great cerebral veins. Tentorial tears may be bilateral and haemorrhage occurs into the subdural space either above or below the tentorium. In such cases, the head of the child shows evidence of well-developed moulding and caput succedaneum. Fractures and dislocations of the limb bones and clavicles may be found.

(2) PROLAPSE OF THE CORD OR PRESSURE ON THE CORD: They produce death by asphyxia. The cord is liable to be compressed by the foetal head, especially in breech presentations. On post-mortem examination, blood, meconium, liquor amnii or vernix caseosa may be found in the bronchial tubes.

(3) TWISTING OF THE CORD ROUND THE NECK OR KNOTS OF THE CORD: It causes compression but no abrasions or ecchymoses and cause death by strangulation.

(4) INJURIES TO THE MOTHER : Heavy blows or kicks on the mother's abdomen or falls from a height may cause concussion of the brain of the child with or without fracture of skull or rupture of blood vessels or organs. Rarely, powerful uterine contractions may fracture the cranial bones of the foetus.

(5) DEATH OF THE MOTHER : The child can be saved if it can be delivered within five to ten minutes of the mother's death.

(B) AFTER BIRTH : (1) SUFFOCATION : It may result when the membranes cover the head during birth, or if the face is pressed accidentally in the cloth or submerged in the discharges, such as blood, liquor amnii or meconium. A child can sruvive in the membranes for 20 to 30 minutes.

PRECIPITATE LABOUR : Labour terminating in a very short time than that taken on the average, either in a primipara or multipara is called precipitate labour. In this delivery occurs suddenly and rapidly without the knowledge of the mother. All the three stages of labour are merged into one. The foetus is normal or premature. It is possible in multiparae with large roomy pelves, but is extremely rare in primiparae. A woman may be delivered unconsciously during fits or periods of coma, hysteria, hypnosis, under the influence of narcotic drugs, anaesthetics, and even deep drunkenness. It is highly improbable that any primiparous woman would be delivered during ordinary sleep without being aroused. Sometimes, a woman may not be able to distinguish the sense of fullness produced by the descent of a child from the feeling of bulky evacuation. The child may die from (1) suffocation by falling into a lavatory pan, (2) head injury and fracture of the skull with

subdural haemorrhage often bilateral, by a fall on a hard floor, if the woman was standing, and (3) haemorrhage from the torn end of the cord.

If the birth occurs in the toilet bowl or into a bucket containing liquid, the infant will inhale the liquid and blood, and meconium and vaginal mucus are found in the air-passages. Microscopic examination of the lungs will show the foreign particles contained in the drowning fluid. In accidental falls, the haemorrhages are usually subdural and often bilateral. The average length of the cord is 50 cm. which is not sufficient to allow the child to fall to the ground, and is sufficiently strong to withstand the weight of the foetus without breaking. The cord is torn most commonly at the foetal end than the placental end, but is not torn in its middle. Caput succedaneum and moulding of the head are absent. Foreign materials, such as mud, sand, gravel may be found in the hair or injured scalp of child. The fractures of the skull are usually fissured and limited to parietal bones, but may extend to frontal and squamous part of temporal bones. Fractures due to forceps lie at points normally gripped by the instrument and are usually "gutter" or "pond" type.

Medico-legal Importance : (1) The mother or her relatives may be accused of killing the infant, while the death may be due to injury, haemorrhage or asphyxia from precipitate labour. (2) In a case of murder, death of the child may be attributed to precipitate labour.

(II) **Criminal Causes :** These may be (1) acts of commission, and (2) acts of omission.

(A) **Acts of Commission :** They are acts done positively to cause the death of the infant. Numerous injuries may be found on the body, especially around the face, head and neck, due to attempts at self-delivery. There may be multiple circumferential abrasions around the whole surface on the neck caused due to fingernails. They should not be mistaken for homicide.

(1) **Suffocation** : The child's nose is closed with two fingers and the lower jaw is pushed up with the palm to occlude the airway. Other methods are placing a pillow or towel over the child's face and pressing down, or pushing the face down into bedclothing. The amount of force to produce smothering is so minor that there is no evidence of trauma. Overlaying, or forcing mud, rag or cotton-wool into the mouth are other methods.

(2) **Strangulation**: Throttling or strangulation by ligature is also common, and in the latter case the ligature is frequently left *in situ*. Sometimes, umbilical cord is used as a ligature to simulate accident. Abrasions on the neck may be caused by the frantic efforts of the mother to deliver herself.

(3) **Drowning**: It is rare, but the body of a dead foetus may be thrown into a well, tank, etc.

(4) **Burning** : Infanticide by burning is rare, but it may be used as a mode of disposal.

(5) **Blunt head injury** :Dashing the head against a wall or the floor by holding the feet is rare. In such cases, there may be bruising of the ankles and feet, where they were firmly gripped. Blows on head may be produced with a blunt weapon. Subdural and subarachnoid haemorrhages are common and are usually accompanied by fractures (depressed or comminuted) of the skull and contusions and lacerations of the brain and scalp. In infants, extradural haemorrhages are limited to single bones because of the adherence of the dura to the skull along the suture lines.

(6) Fractures and dislocation of cervical vertebrae: These may be caused by twisting the neck.

(7) Wounds : The child may be killed by stabs, incised wounds, cut-throat, etc.

(8) Poison : Rare

(B) Acts of Omission or Neglect : A woman is guilty of criminal negligence, if she does not take ordinary precautions to save her child after birth. The following acts of omission amount to crime. (1) Failure to provide proper assistance during labour

Table (19–2). Difference between head injury due to labour and blunt force.

Trait	Head injury due to labour	Head injury due to blunt force
(1) Bruises :	May be present on the presenting parts of the scalp.	Found anywhere on the scalp.
(2) Lacerations :	Not present on the scalp.	Present on the scalp.
(3) Fractures :	Fractures are fissured; usually of parietal bones and run downwards at right angles to the sagittal suture.	Extensive comminuted and depressed fractures of the skull bones affecting vault or base.
(4) Brain :	Usually not injured.	Contusions, lacerations and haemorrhage.

may cause death by suffocation or head injury. (2) Failure to tie the cord after it is cut may cause death by haemorrhage. (3) Failure to clear the air-passages which may be obstructed by amniotic fluid or mucus. (4) Failure to protect the child from exposure to heat or cold. (5) Failure to supply the child with proper food.

THE ABANDONING OF INFANTS : If the father or mother of a child under the age of twelve years, or anyone having the care of such child, leaves such a child in any place with the intention of abandoning the child, shall be punished with imprisonment up to seven years (Sec. 317, I.P.C.).

CONCEALMENT OF BIRTH : Whoever, secretly buries or otherwise disposes of the dead body of child, whether such child dies before or after or during its birth, intentionally conceals the birth of such child, shall be punished with imprisonment up to two years (Sec. 318, I.P.C).

CHILD ABUSE: Alcoholic and drug addicted parents are more likely to abuse or neglect their children.

BATTERED BABY SYNDROME OR NON-ACCIDENTAL INJURY OF CHILDHOOD: It is also known as child abuse syndrome, Caffey's syndrome, and maltreatment syndrome in children. The typical form of this condition is very rare in India. A battered child is one who has received repetitive physical injuries as a result of non-accidental violence, produced by a parent or guardian. In addition to physical injury, there may be non-accidental deprivation of nutrition, care and affection. The classical features of syndrome are obvious discrepancy between the nature of the injuries and explanation offered by the parents, and delay between the injury, and medical attention which cannot be explained. The constant feature is repetition of injuries at different dates, often progressing from minor to more severe.

Features : (1) **Age** : Usually less than three years old, though it may occur at any age. (2) **Sex**: Slightly more in males (55 to 63%). (3) **Position in family**: One child of a family, commonly the eldest or the youngest and often unwanted, such as the result of pregnancy before marriage, failure of contraception or an illegitimate child. (4) **Socio-economic factors**: Parents tend to be young between 20 to 30 years, and belong to lower social class and lower education. The family is usually isolated. There is often a history of family disharmony, long-standing emotional problems or financial problems. Many of the fathers have criminal records, or unemployed or socially unstable. Many mothers have multiple social and psychiatric problems with a chaotic and violent home background. The mother is of lower I.Q., often pregnant or in the premenstrual period at the time of battering. Unhappy childhood experiences are common in both parents and many battering parents were "battered children" themselves. Most of the parents suffer guilt-amnesia. (5) **History**: There is obvious difference between the nature of the injuries and the explanation given by the parents, which may change on several times of repetition, each time the child is taken to a different doctor. (6) **Treatment** : There is always delay between the injury and medical attention. (7) **Precipitating factors** : Violence is precipitated by actions of the child itself, e.g., crying, refusal to be quiet, persistent soiling of napkins, etc.

Injuries : Direct manual violence is the commonest method of injury. **Surface injuries** : Soft tissue injuries are very common and may be seen almost anywhere on the child's body. The head, face and neck show bruises, abrasions and lacerations of different ages. Multiple bruises are seen on brows, cheeks, mouth and neck. Laceration of the mucosa inside the upper lip, often tear of the fraenulum is the most characteristic lesion. This may extend laterally and separate the inner surface of the lip from the base of the gums. This injury results from a blow on the mouth or due to other efforts to silence a screaming or crying child. Multiple bruises of various ages all over the body from rough handling, beating, kicking or throwing the infant are common. Bruises may be seen on either side of the chest, behind the axillae and down the anterior chest wall, where the child has been gripped roughly, between two adult hands and shaken. Caffey (1974) described the effects of shaking a child as a major cause of subdural haematoma and intraocular bleeding in battered babies, the so-called" **infantile whiplash syndrome**". Recent research has thrown doubt on the common acceptance of this mechanism. In such cases, bruises are produced in areas where the child is held by the hands, but there are no external injuries to the head or fractures of the skull, but there may be traction lesions of the periosteum of the long bones without fracture. Permanent brain damage may be caused due to habitual, prolonged shaking. Bite marks may be found on the cheeks, shoulders, chest, abdomen, arms, legs and buttocks. Bruises are usually present around the elbows and knees due

to gripping of the child, so as to shake or pull him, or hurl him into cot or against furniture, etc. Slap marks may show clear lines of petechial haemorrhages. Knuckle punches show as rows of three or four roughtly round bruises. Bruising caused by belts, straps, canes, pieces of wood, hair brushes may be seen frequently on the buttocks and thighs. Pinch marks may appear as butterfly-shaped bruises with one wing caused by thumb larger than the other. Subgaleal haematoma resulting from vigorous pulling on the scalp is characteristic. Bald patches on the scalp due to pulling out the hair (traumatic alopecia) is very characteristic. **Eye:** Retinal separation, lens displacement, retinal haemorrhages, vitreous haemorrhages, subconjunctival haemorrhages, and subhayaloid haemorrhages and black eye have been found. **Visceral Injuries** : Subdural haemorrhage is found in about 40% of fatal cases.Crushing or compressing force applied to the abdomen produce either "bursting" injuries of the liver or spleen, or perforations of distended hollow viscera including the stomach, intestine or urinary bladder. The second part of the duodenum and jejunum may be completely transected. Deceleration or whipping froces produced by punches or blows tear the mesentery and can lead to disruption of the small intestine. Extensive internal injuries may be present with minimal external signs of injury. **Burns** : Stubbing of cigarette ends upon the skin produce small circular, pitted burns which are pink or red when fresh. When healing, they tend to be silvery in the centre with a narrow red rim. The child may be made to sit upon a hot stove or electric radiator or he may be dipped in very hot fluids. **Skeletal Injuries** : Large periosteal haematomas are common because periosteum is readily stripped in infants. Bleeding under the periosteum causes calcification, which is seen on X-ray as an extra line of opacity running alongside the affected length of bone. The violent forces applied to the limbs involve pulling and twisting, both capable of producing epiphyseal separation and periosteal shearing. Transverse and spiral fractures of long bones result from compression, bending and direct forcible blows. Anteroposterior compression of the chest causes fractures of ribs in midaxillary line. Violent squeezing of the chest from side to side causes fractures at the costochondral junctions. Multiple rib fractures also occur along the posterior angles of the ribs. After one to two weeks, callus is formed, and on X-ray" a string of beads " appearance is seen in the paravertebral gutter (**Nobbing fractures**). Avulsion of the metaphysis or chipping of the edges of the metaphyses or epiphyses may occur, with small fragments seen isolated on X-ray. Before autopsy, a whole body X-ray should be taken to detect old fractures and especially metaphyseal and epiphyseal injuries in various stages of healing.

Diagnosis :The diagnosis depends upon (1) nature of injuries, (2) time taken to seek medical advice, and (3) recurrent injuries. Differential diagnosis has to be made from scurvy, congenital syphilis, osteomyelitis, leukaemia, rickets, juvenile osteoporosis with stress fractures, paralytic disease with fractures, infantile cortical hyperostoses and osteogenesis imperfecta. Radiological manifestations of trauma and especially the metaphyseal lesions are specific to the battered baby syndrome.

MUNCHAUSEN'S SYNDROME BY PROXY: Munchausen syndrome is feigning illness or injury and going from hospital to hospital for unnecessary investigations and treatment. Munchausen's syndrome by proxy is a variation which is a peculiar and dangerous type of child abuse usually involving the mother, in which children are brought to doctors for induced or fabricated signs and symptoms of illnesses with a fictitious history. The sex ratio is almost equal. It has been described in children of few weeks of age to 21 years. The child is admitted frequently in the hospital for medical evaluation for the non-existent conditions. These patients appear to be compulsively driven to make their complaints. The person is aware that he is acting an illness, but he cannot stop the act. There is continuity, ranging from exaggerated claims of infirmity to actual selfinduced illness. At the extreme end, life-threatening injuries are masqueraded as being legitimately contracted. Rosenberg (1989) gave four diagnostic crteria: (1) Illness produced or alleged, or both by a parent. (2) Repeated requests for medical care of a child, leading to multiple medical procedures. (3) Parental denial of knowledge of the cause of symptoms. (4) Regression of symptoms when the child is separated from the parents.

Method of simulation or production of illnesses: (1) The mother pricks her finger and adds blood to the urine of the child and takes the sample to the doctor. (2) The child's nose is closed with two fingers and the lower jaw pushed up with the palm to block the airway. (3) A pillow or towel is put over the face of the child and the face is pushed down into bed clothing.(4)The mother gives

insulin to the child and takes to hospital with hypoglycaemia. (5) Vomiting: allegation or by ipecacuanha. (6) Diarrhoea: laxatives, salt poisoning. (7) Convulsions: allegation or by theophylline, insulin, psychotropic drugs. (8) Bleeding: anticoagulants, phenolphthalein poisoning, exogenous blood. (9) CNS depression: barbiturates, benzodiazipines. (10) Fever: alleged. (11) Rash: scratching or intoxication.

SUDDEN INFANT DEATH SYNDROME

Sudden infant death syndrome (SIDS), or cot death or crib death is defined as the sudden and unexpected death of seemingly healthy infant, whose death remains unexplained even after thorough case investigation, death scene examination, review of clinical history and complete autopsy.

Features : (1) Incidence : 0.6 per thousand livebirths. (2) **Age :** 2 weeks to 2 years, but most deaths take place between one and 7 months, with a peak at 2 to 3 months. (3) **Sex :** There is slight increase in males (4) **Twins :** There is increased risk (threefold) amongst members of a twin pair. Most twins are premature and of low birth weight. (5) **Geographical distribution :** The occurrence is worldwide. (6) **Time of death :** Death always occurs during sleep at all times of night with a moderate increase in the early morning hours. (7) **Prematurity** has a higher risk. (8) **Socio-economic** standard of the family is usually low. (9) **Cigarette smoking and drug abuse** by pregnant women increase the risk.

The child is either quite well when put to the bed, or may have only a minor upper respiratory tract infection (cold or snuffles), or minor gastro-intestinal disturbance. Cot deaths are major cause of death in infants in the first six months of life.

Autopsy : Milk or a blood-stained froth is sometimes seen on the child's mouth, nostrils or bedding. The post-mortem findings are negative. In about 15% of cases, some pathological condition may be found, such as frank pneumonia, congenital heart disease, Down's syndrome or a tracheobronchitis. The only constant findings are multiple petechial haemorrhages on the visceral surfaces of the heart, lungs and thymus (70 to 75%) which are agonal in nature, perhaps from terminal respiratory efforts against a closed glottis. A small amount of milky vomit in the trachea and main bronchi, and shedding of individual tracheobronchial epithelial cells are commonly found. Many infants show froth in the air-passages and facial pallor. There are no petechial haemorrhages in the face or eyes. The hands are often clenched around fibres from the bed clothes. The lungs show patchy or uniform purplish discolouration of the surface and are firm in consistency with congestion, oedema, patchy alveolar collapse and increase in weight. The alveolar walls are thickened and are infiltrated with lymphocytes and occasional neutrophils and monocytes. Peribronchiolar cell infiltration is the main finding. Laryngitis, tracheitis, bronchitis, bronchiolitis, pneumonitis and pleuritis either individually or in various combinations may be found. In the majority of cases, the extent of the pathology present is rarely sufficient to cause death.

Theories : Various theories have been advanced, but there is no single cause of cot death, and death may result from a number of causes which combine fatally while a child is passing through a vulnerable period of development. Some infants have prolonged **"sleep apnoea"** (a periodic failure to breathe during sleep), which makes them susceptible to hypoxia, which finally leads to bradycardia and cardiac arrest, but this has not been substantiated. Respiratory infection may produce a viraemia which adds to the sleep depression of the respiratory centres. Nasal oedema and mucus secretion may further narrow the small upper respiratory passages and in some hypotonic babies, a flaccid pharynx and even neck posture may further reduce the airway. An element of laryngeal spasm has also been suggested. Whatever the cause, factors in pregnancy that inhibit foetal circulation could damage the child's brain, so that it no longer controls breathing properly. An unidentified trigger could affect the airway of a sleeping infant. The brain would not respond correctly and breathing would stop. Staphylococcus aureus infection of upper respiratory tract is said to cause anaphylactic shock and sudden death.

Other causes of death which have been proposed are conduction system anomalies, mechanical upper airway obstruction due to anatomical abnormalities, respiratory viral infection, adrenal insufficiency, gastro-oesophageal reflux leding to bradycardia, liver enzymes, hypersensitivity to cow's milk, deficiency of parathyroid, selenium, antibodies, calcium, vitamin D,E,B, magnesium, etc., house-mite allergy, anaphylaxis, sodium overload in feeds, hyperthermia, hypothermia, suffocation by bed clothes and pillows, bacterial infection, neurogenic shock, hypogammaglobulinaemia, metabolic disorders, etc..

CHAPTER 20
BLOOD STAINS

All kinds of stains should be sent to the State Forensic Science Laboratory for examination. The source of the blood (human or animal) is determined by the Serologist of the Government of India at Calcutta. The stained article is allowed to dry at room temperature. No extra heat should be used as this will cause deterioration of the stain. If the stained clothes are not dried, putrefaction sets in, and it becomes difficult or impossible to know whether the blood is of human or animal origin. The stained object should be identified by initialling the object and dating it. If this is not possible, a tag or other device will have to be identified and attached.

Collection of Blood Stains: (1) A clean piece of white filter paper may be used, allowing blood to soak into it, then drying it at room temperature. A control filter paper should also be sent for examination. (2) If the object is porous, a portion of unstained area should also be taken. (3) If the object is non-porous and particularly if it is metallic, stains can be removed by scraping and placed in small glass containers. They should not be placed in envelopes where they will be reduced to powder. (4) Stains on clothing may be scraped off or a fragment of the material cut.

The solvents for blood stains are: (1) 10% solution of potassium cyanide. (2) 10% solution of glycerine in distilled water. (3) A weak solution of ammonia. A coloured solution is obtained immediately with any of the above solvents. Otherwise, the material must be covered and left for from 12 to 24 hours at room temperature.

Stains on Clothing: In the case of clothing, type of garment, its colour and consistence should be noted and if the garment is torn, the position of the tears should be noted. Whether the clothes were dry, damp or wet when received should be noted. Both the outer and inner surfaces of the garments should be examined. The position of all stains should be given correctly by a description of the stain in its relation to the manner in which a garment is usually worn, e.g., a stain on the trousers should be described as being above, behind, or to the outer side of the knee. Stains may also be described in relation to the pockets, the buttons, or the seams of a garment. The size and the shape of the stain should be noted. If the stain is in the form of a smear, its general direction should be noted. Blood stains are extremely resistant to washing by water. The dried blood on a dead body or article will remain intact for quite a long time, even though the body has been totally submerged. Invisible blood stains can be detected by spraying luminal on the cloth or stained material with an atomiser inside a dark room. The stained area will luminesce if blood is present in those areas.

SUBSTANCES RESEMBLING BLOOD STAINS

(1) RUST STAINS: On knives and steel weapons they resemble dried blood stain, but they do not have a dark and glazed appearance and do not fall off in scales, when the opposite side of the blade is heated. They do not stiffen the cloth, and are soluble in dilute hydrochloric acid. Tests for iron are positive.

(2) SYNTHETIC DYE STAINS: They are changed to yellow by nitric acid, and the original colour is restored by a strong solution of alkali.

(3) MINERAL STAINS: They generally contain oxides of iron, red lead or red sulphide of mercury.

(4) VEGETABLE STAINS: Certain fruits, e.g., mulberry, gooseberry, currants, jambans, etc., produce stains which resemble blood stains. On microscopic examination, vegetable cells and detritus are seen. Henna, catechu, *pan juice*, tobacco, and the barks, leaves and fruits of some trees produce red stains resembling blood stains. Most of them contain tannin, which becomes black if a drop of ferric chloride is added.

(5) OTHER STAINS: Spots of grease, resin, tar and pitch especially on dark fabrics may resemble old blood stains. The tests for blood are negative.

EXAMINATION OF BLOOD STAINS

(1) GENERAL: (1) Stains found at the scene of the crime: One of the important aspects of the visit to the scene of crime is searching for and interpretation of bloodstains. Relatively minor blood smearing may also provide significant evidence, such as a smear on the door handle. Heel prints or shoe prints on bloodstained area of the body, help in the identification of the assailant. The distribution and amount of blood at the scene of the crime may give valuable information about the manner of death, whether it was suicidal or homicidal, and whether the victim struggled or moved about after his injuries. The vessels usually go into

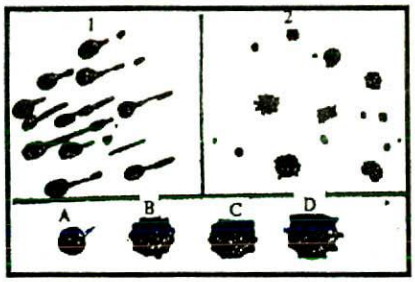

Fig. (20–1). (1) Spurting (indicating direction).
(2) Blood falling vertically.
(3) Drops of blood from various heights.

spasm for a second or two after the injury and therefore the site of blood staining may not be site of infliction of injury. When much blood is present, it suggests serious injury during life, but if a large vessel is cut, bleeding can occur after death. The collection of a pool of blood near the body during life indicates that the deceased fell unconscious and remained immobile after the injury. Usually the amount of blood present at the scene cannot be measured, and the amount present is likely to be overestimated because of the highly coloured nature of blood. A trail of blood stains will indicate that the victim was wounded at some distance from the place at which the body is found. The victim's person and clothing show dried blood streaks running from the wounds towards his feet. It can happen when the victim is attacked while running or in case of suicide. If a victim is injured while he was on the floor, the dried blood streaks usually run down his sides. Blood coming from the arteries of a living person will be scattered in a fine spray over surfaces upon which it has fallen. A major vessel, such as the common carotid when cut can spray blood up to a distance of half a metre. Blood spray at the scene of violence is usually due to waving of the weapon, rather than direct spray from blood vessels. Venous bleeding is a slow steady flow, causing a pool if the victim is at rest, and separate widely spaced drops, if the victim walks about. The shapes adopted by blood spots may be drops, smears, splashes, spurts, trails, and pools.

The direction of the fall of blood on to a surface may be recognised. If it drops vertically on to flat surface, the stains are circular and the pattern is distinct according to the distance travelled. If the height does not exceed a few centimetres, the drop appears as a round spot. If it has travelled thirty cm. or more, it shows prickly edges, the projections growing finer and larger in number with the increase in length. When the height is still greater, ray-like splashes break out from the drop, and may be seen up to a distance of 15 to 20 cm. Splashes of blood striking a surface obliquely may appear like spears or exclamation marks, depending on the velocity and angle of the fall; the pointed end indicates the direction of the motion. If projected on to a wall by an upward sweep of an injured hand, the dots point upwards; or if by a downward sweep, downwards. If the long axis of the stains lie horizontally, it is possible to tell whether the drops fell in a forward or backward direction. Discontinuous spots of blood on the walls of the room indicate splashing of blood from the victim, the assailant, or the weapon, or spurting of blood from a cut artery. Blood which has run down a surface before clotting shows thickening of clot at its lower margin. When blood falls upon porous articles or clothing, such as linen or cotton, it is absorbed, and spreads. It may be possible to see clear drag marks. Smears caused by fingers or palms are helpful in identification. A photograph of blood stains at the scene of a crime is useful.

(2) PART OF THE BODY FROM WHICH STAIN IS DERIVED? Menstrual blood is usually found on female garments, diapers or pieces of cloth. It is dark and fluid, has a disagreeable smell and the reaction is acid. On microscopic examination it shows endometrial and vaginal epithelial cells, and number of microorganisms consisting of groups of bacilli and cocci. Trichomonas vaginalis or monilia may be present. It contains fibrinolysins. If the blood is from the NOSE, mucus and hair from the nose may be found. VOMITED BLOOD is of chacolate colour and acid in reaction due to the action of gastric juice. Blood due to HAEMOPTYSIS is bright red and frothy, with alkaline reaction. In blood due to RAPE, semen and pubic hair may be found. Blood stains due to BOILS AND SORES show a smeared appearance without definite drops of blood, and may contain pus cells and bacteria.

(3) AGE OF BLOOD STAINS: Fresh stains on light coloured clothes are of bright-red colour, which gradually changes to reddish-brown in 24 hours, and brown within a few days, which may become black after a long time. Fresh stains are moist and sticky, and on drying, they stiffen the cloth because of the proteins. On many metallic articles, blood stains appear as dark shining spots or smears, and when dry, show fissures and cracks. In ordinary conditions, a drop of blood dries in an hour or two. If blood is collected in pools, it may take 12 to 36 hours to dry, depending upon the size and depth of the pool formed. The recently shed arterial blood is bright-red and venous blood dark-red. The solubility of blood stains in water and other liquids depends mainly on the age

of the stains and the type of material on which it is found. The fresher the blood, the more easily it is dissolved. The solubility gradually diminishes with age. The age can also be determined by the spread of the soluble ingredients like chlorides into surrounding material. Fluorescence decreases as the stain becomes older due to the increasing amount of haematin. It can only be stated that the stain is very fresh, recent, some weeks, months, or very old.

(4) SEX AND AGE OF PERSON: Sex can be determined from the presence of sex chromatin in the leucocytes, if the cells can be identified. At birth, the blood forms a thinner and softer coagulum. The presence of foetal haemoglobin indicates that the blood is derived from a child.

(5) LIVING OR DEAD BODY: Blood which has effused during life can be removed in scales on drying, due to the presence of fibrin. Blood which has flowed after death tends to break up into a powder on drying.

(6) SOURCE OF BLOOD: If the victim and assailant are of different blood groups, it is helpful in establishing the identity. If the stains are on the inner side of the garment, they usually belong to the victim, but if found outside they may belong to the victim or accused.

(II) CHEMICAL EXAMINATION: The chemical tests depend on the presence in the blood stains of an enzyme peroxidase, which in the presence of hydrogen peroxide, oxidises the active ingredient of the reagent and produces the characteristic coloured compound.

(1) Benzidine Test : Cut out a small piece of stained material or tease out fibres from the stained fabric and place it on porcelain tile. Add a drop of saturated solution of benzidine in glacial acetic acid, and then a drop of 10 volumes hydrogen peroxide. If blood is present, dark blue colour is produced immediately.

A positive reaction is given by blood of almost any age, blood that has been exposed to heat or cold. and blood stains treated with cleaning agents. This is the best preliminary test for blood and it detects blood when present in a dilution of one part of blood in three lakhs. A positive reaction is not proof of the presence of blood, but a negative reaction rules out of the presence of blood. A weaker reaction is obtained from certain other substances, e.g. pus, saliva, milk, rust, formalin, certain vegetable and animal juices, oxidising agents, bacteria, etc.

(2) Phenolphthalein Test (Kastle-Meyer Test): To a solution extracted from the stain with distilled water, add ten to twenty drops of phenolphthalein reagent (phenolphthalein 2g. + sodium hydroxide 20g. + zinc+ distilled water 100 ml), and then a drop or two of 10 volumes hydrogen peroxide. If blood is present a pink or purple colour develops immediately. The test is more specific for blood than benzidine test, but comparatively less sensitive. Traces of copper give positive reaction.

The tests employing guaiacum (deep blue) and leucomalachite green are rarely used in medico-legal work.

(III) MICROSCOPICAL AND MICROCHEMICAL EXAMINATION

(1) Red Corpuscles: Intact red cells are seen only when the stains are fresh or when a blood clot is available. The red cells become unrecognisable when dried. A small piece of stain is cut out and soaked in a watch glass with two or three drops of Vibert's fluid (sodium chloride 2 g., mercuric chloride half g., distilled water 100 ml.), or normal saline for half hour. It is then teased with needles and examined under high power. If the stain is not dissolved, dilute solution of ammonia or 2% hydrochloric acid can be used. Red blood cells are circular, biconcave, non-nucleated discs in all mammals except camels. In camels, they are oval and biconvex but non-nucleated. In birds, fishes, amphibia, and reptiles, they are oval, biconvex and

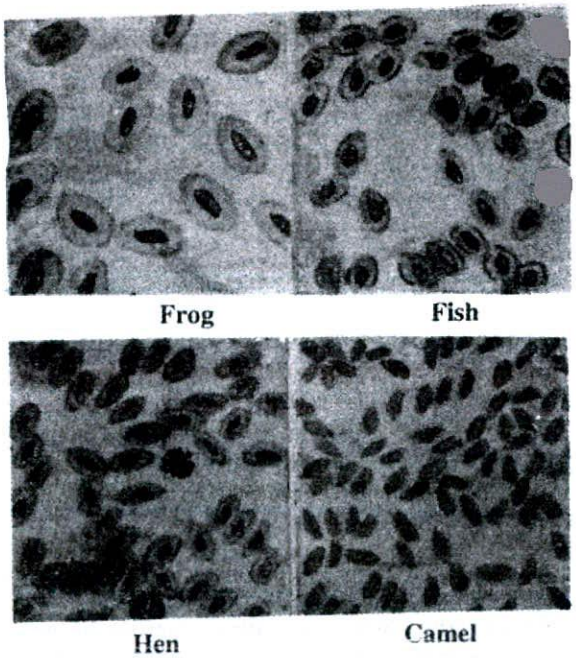

Fig. (20–2) Red blood corpuscles.

nucleated. Pus cells, epithelial cells, bacteria, faecal matter, etc., are sometimes found mixed with blood.

(2) Haemin Crystal Test (Teichmann's test): A small crystal of sodium chloride and two to three drops of glacial acetic acid are placed on a small piece of the suspected stain on a glass slide. A coverslip is applied and the acid is evaporated by heating over a small flame. It is examined under the microscope after cooling. Faint yellowish-red to brownish-black rhombic crystals of heamin or haematin chloride arranged single or in clusters are seen if blood is present. Bubbles of gas are given by haematin crystals, on addition of a drop of hydrogen peroxide. The reaction is negative if the stain is old, is washed or treated by chemicals, presence of too much salt, moisture in acid and over-heating.

(3) Haemochromogen Crystal Test (Takayama test): Place a small piece of suspected material on a glass slide and add 2 to 3 drops of Takayama reagent (sodium hydroxide, pyridine, glucose), and cover with a coverslip. Pink, feathery crystals of haemochromogen or reduced alkaline haematin arranged in clusters, sheaves, etc., appear in one to six minutes. Slight warming of the slide hastens the reaction. The result is negative if crystals are not formed in half hour. The test gives good result even with old stains. It is delicate and more reliable.

Electrophoresis and immunoelectrophoresis can positively identify blood stains. Separation and migration of haemoglobin and its derivatives can be done by chromatography.

(IV) SPECTROSCOPIC EXAMINATION: It is the most delicate and reliable test for detecting the presence of blood in both recent and old stains. Less than 0.1 mg. of blood is sufficient. The blood stain is dissolved in water, normal saline or dilute ammonia, and is placed in a small glass test tube which is then kept between the spectroscope and the source of the light. The extract of the blood must be dilute and if turbid it should be filtered. The solution of the blood has the property of absorbing some of the rays from the spectrum, producing characteristic dark absorption bands, which vary with the type of the blood pigment present.

SPECTRA OF HAEMOGLOBIN AND ITS DERIVATIVES: (1) OXYHAEMOGLOBIN is marked by two distinct bands in the yellow between the Fraunhofer lines D and E, the one nearer D being about half the breadth of the other and more defined.

(2) REDUCED HAEMOGLOBIN shows a broad band which lies between D and E.

(3) CARBOXYHAEMOGLOBIN has a spectrum similar to oxyhaemoglobin which remains unchanged after addition of ammonium sulphide, which reduces oxyhaemoglobin.

(4) METHAEMOGLOBIN spectrum is similar to oxyhaemoglobin with third dark band in the red between C and D, and the fourth between E and F, which is more indistinct.

(5) ACID HAEMATIN has a sharp band between C and D and a broad band between D and F which is not well-defined.

(6) ALKALINE HAEMATIN has a band between C and D.

(7) HAEMOCHROMOGEN or reduced alkaline haematin has a dense narrow band midway between D and E and a pale, broad band over E.

(8) CYANHAEMOCHROMOGEN has absorption bands, similar to those of haemochromogen, but slightly wider.

(9) ACID HAEMOTOPORPHYRIN gives a dark sharp and broad band between D and E.

(10) ALKALINE HAEMATOPORPHYRIN consists of four bands. One between E and F is darkest and broadest, two are between D and E, and the fourth between C and D.

(V) SEROLOGICAL EXAMINATION: This

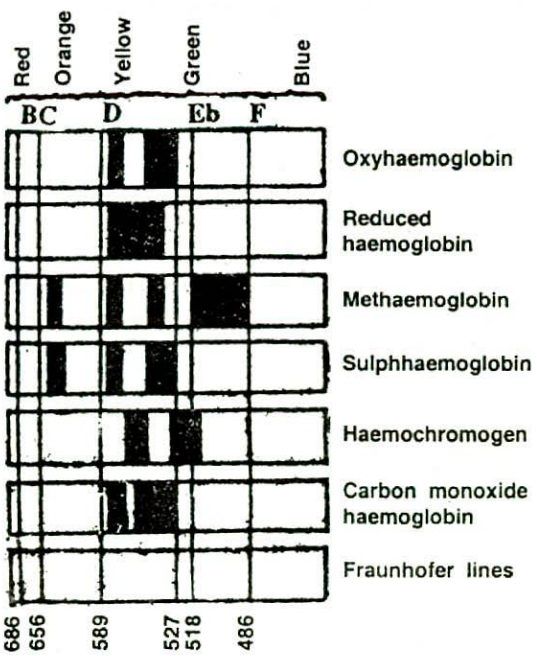

Fig. (20–3). Absorption spectrum of haemoglobin and its derivatives.

determines whether the blood is derived from human being or from a lower animal.

(A) Immunological Methods:

(1) Precipitin Test: Principle: Blood serum contains proteins in colloidal suspension, and when human serum is injected into an animal, the animal becomes immunised against these proteins and antibodies develop in its blood. If human serum is then brought into contact with this animal serum, the antibodies in the animal serum react with the proteins in the human serum and a visible precipitate forms. The antibodies causing this reaction are known as precipitins and the animal serum is known as antihuman precipitin serum. A rabbit or a fowl is injected with human blood every third day for 3 to 5 injections. After this the animal is killed and the antiserum is collected. A suitable antiserum should react immediately or within a minute on the 1 : 1,000 dilution.

Technique : The presence of blood is determined first. An extract of stained material is prepared by soaking it in normal saline. No chemical should be added to extract the stain. The extract should be clear and may be filtered or centrifuged if necessary. The extract is diluted to 1: 100 with normal saline. Two drops of undiluted antiserum are gently added to 0.75 ml. of diluted stain extract in a small tapering test tube held in a slanting position. The antiserum slowly settles down to the bottom, and at the junction of two fluids, a white ring with well-defined borders appears in the case of a positive reaction. The ring is situated mostly in the antiserum. In the case of negative reaction no ring appears. A positive reaction should begin in ten minutes and should be read in half hour. The results are qualitative and expressed as positive or negative. For medico-legal purposes all doubtful reactions are read as negative.

Application of the Test: It is a specific protein test, and the reaction demonstrates the presence of albuminous substances obtained from any part of human body. The origin of skin, flesh, bone or even secretions, such as saliva, milk and semen is determined by this test.

(2) ANTIGLOBULIN CONSUMPTION TEST: (Haemagglutination inhibition test): When human globulin is mixed with antihuman globulin serum, the latter is absorbed and is no longer capable of agglutinating Rh positive red cells sensitised with incomplete anti-D. This detects globulins in dilutions of 1 : 50,000

(3) GEL DIFFUSION: Wells are punched in an agar plate and antiserum and antigen are placed in adjacent wells. Diffusion will occur and precipitin bands will develop between wells containing corresponding antigen and antiserum. The advantage of this method is that there is no necessity to obtain clear antisera and stain extracts.

(4) DOUBLE DIFFUSION IN AGAR GEL: A piece of stained material or extract is placed in a central well cut in agar gel, while each of six wells surrounding this contains a drop of antiserum specific for the globulin of a particular species of animal. The advantage of this method is that the extract can be tested against different antisera simultaneously.

(5) PRECIPITATION-ELECTROPHORESIS: The gel is poured on microscopic slides in a layer one to two mm. thick, and wells punched after it has solidified. The extract of the stain is placed in the cathodic well and the antiserum in the anodic well. After 15 to 20 minutes of electrophoresis precipitin band will be visible between the wells where positive reactions have occurred.

(6) LATEX TEST: A saline extract of blood stain is mixed with dilute suspension of latex particles sensitised with antiserum. A positive reaction is shown by agglutination of the particles into clumps.

(B) ISOENZYME METHODS: These are based on the electrophoretic demonstration of the existence of enzymes in blood of the same species in multiple molecular forms known as isoenzymes. These methods are relatively less sensitive than immunological methods.

Blood groups ABO, MNS and Rh are determined in a blood stain by (1) Latte's crust method. (2) Absorption-elution technique. (3) Absorption-inhibition. (4) Latex method. (5) Enzymological methods.

Medico-legal Aspects of Blood Groups: The application of blood groupings to medico-legal problems is based on the following principles: (1) A blood group antigen cannot appear in a child, unless present in one or other parents. (2) If an individual is homozygous for a blood group factor, it must appear in the blood of all his children. (3) If a child is homozygous for a blood group factor, the gene for the same must have been inherited by it from each of its parents. (4) The blood group characters are peculiar to the individual and are unchanged throughout life.

EXCLUSION OF PATERNITY: **(1) First-order exclusion: Where the child has a blood group gene that is absent in both the mother and the putative father. (2) Second-order exclusion. Where the putative father is homozygous for a blood group gene, but the gene**

is not present in the child in question.

Phenotype is the entire physical, biochemical and physiological make-up of an individual as determined both genetically and environmentally.

AGGLUTINOGENS: The red cell antigens, representing over 400 serologically determined specificities, belong to a large number of blood group genetic systems with individual chromosomal loci. They are situated in the outer envelope of the red blood cells. They appear in the cells early in foetal life. They are also called isoagglutinogens or antigens.

AGGLUTININS: Isoagglutinins appear in the latter half of antenatal life. Agglutinins at birth may be derived from the mother by filtration through the placenta. They diminish or disappear during the first ten days of life, after which the infant p oduces its own agglutinins.

BLOOD GROUPS : Blood group antigen is attached to cell membrane of red cells, which appear early in foetal life. Agglutinins appear in the latter half of antenatal life. Landsteiner (1930) discovered ABO blood groups. The presence of blood group substances is shown by a clumping (agglutination) of the red cells when they are mixed with appropriate antisera. The antisera are prepared from human blood which contain agglutinating bodies called antibodies which will detect the antigens on the red cells.

The genes which are present in the nuclei of all cells of the body control the formation of the antigens. On Mendelian principle, a person inherits one gene for each blood group from each parent, who themselves may be homozygous or heterozygous for the particular group. If the genes contributed by each parent are the same, the individual is called homozygous for the particular gene and if different, he is heterozygous.

The blood group systems currently in use are: (1) Red cell antigens. (2) White cell antigens. (3) Serum protein polymorphisms. (4) Red cell enzyme polymorphisms.

(I) RED CELL ANTIGENS:

(1) THE ABO SYSTEM: Human blood may be divided into four distinct blood groups, A, B, AB and O, depending upon the presence in the red cells of two agglutinogens which are designated by the letter A and B. The A, B and O characters are inherited by means of three allelomorphic genes, every individual having two chromosomes each carrying A, B or O, one from each parent. Thus the possible genotypes are AA, AO, BB, BO, AB, and OO. Group A type may thus be AA or AO. Since there is no true O antigen, group O individuals would be more precisely identified as group H individuals. H–h is inherited independently of the ABO system. The rare hh individuals (Bombay phenotype) lack A, B, or H antigens on their erythrocytes and in ABH substances in most secretions. Antigens of the ABO system can be detected even before birth. Their strength goes on increasing after birth, till about three years of age.

Table (20–1). Determination of ABO groups

Known test sera		Unknown			
Groups	Agglutinins	O	A	B	AB
A	b	–	–	+	+
B	a	–	+	–	+
O	a & b	–	+	+	+

\+ = Agglutination

– = Absence of agglutination.

Table (20–2). ABO blood groups.

Blood group	Agglutinogens	Agglutinins
A	A	Anti-B
B	B	Anti-A
AB	A and B	None
O	None	Anti-A and Anti-B

A and B are both "dominant" to O, and O is "recessive" to A and B, whereas A and B are equally dominant. AA the homozygote, cannot be serologically differentiated from AO, the heterozygote, and the same occurs with the genotypes, BB and BO, the serologically demonstrable blood groups (phenotype) in each case being A and B. The other phenotypes are AB and O. A has two subgroups A_1 and A_2. These are also found in group AB giving rise to subgroups, A_1B and A_2B. Subgroups A_3, A_4 and A_5 are weak and very rare. Anti-A or a and anti-B or b agglutinins are normally developed in the serum against whichever agglutinogens are absent from the red blood cells. There is no positive method of identifying the 'silent' gene O. ABO group is not found in CSF. Group O is universal donor, and AB universal recepients.

Gram-negative bacteria like E.Coli, Proteus, etc. may contain substances similar in property to A, B and O blood group substances and are likely to vitiate the tests.

(2) THE MNSs SYSTEM: Two further agglutinogens M and N, which are quite distinct and independent of the agglutinogens A and B, occur in human blood. The M and N factors are inherited as Mendelian dominants. They are present at birth. They form three groups, M, N and MN. Anti-M and Anti-N agglutinins are not normally developed in human sera but they may be present rarely. The

agglutinogens, M and N are feebly antigenic. The subgroup S is closely allied to the MN system. The phenotypes are: MS, NS, MNS, MNs, MSs, NSs, NNSs.

(3) THE P SYSTEM: P factor is present in about 75 percent of persons, but satisfactory anti-P serum is rare. The main possible genotypes are P_1P_1, P_1P_2 and P_2P_2. P seems to be transmitted as Mendelian dominant. It is weak and medico-legally unimportant.

(4) THE Rh SYSTEM: The Rhesus factor was detected originally by the use of serum from a rabbit which has been immunised against the red blood cells of the Rhesus monkey, and hence the term Rh factor. This factor is present in the red cells of 90 percent of Indians, who are known as Rh- positive, the remainder Rh-negative. Amniotic fluid contains Rh.

A complex of antigens is involved, six in number, named Cc, Dd, and Ee, each of which is capable of producing antibodies, although they are all not equally powerful in this respect. As the gene 'd' is amorphic, 'd' antigen is not produced and the symbol 'd' stands for the absence of D rather than the presence of d. The Rh antigens can be detected in a foetus after six weeks of pregnancy.

A member of each pair occupies one of three closely related genes in the relevant chromosome. Eight combinations are therefore possible, and 36 genotypes could result from them. The antibodies do not occur naturally in human serum. They are produced by the entry of a Rhesus antigen into blood, which is foreign to the individual, either due to blood transfusion or from a foetus in pregnancy.

(5) Ii SYSTEM: The red cells of all adults carry an antigen I, and smaller amounts of the related antigen ii.

(6) KELL SYSTEM: The major antigens are K and k, K_{pa}, K_{pb}, K_o, J_{ka}, J_{kb}, Cote and W_{ka}.

(7) KIDD SYSTEM: Four phenotypes Jk (a+b), Jk (a–b+), Jk (a+b+) and Jk (a–b)– are controlled by two genes Jk^a and Jk^b, which are inherited as co-dominant characters.

(8) DUFFY SYSTEM: The main antigens Fy^a and Fy^b are controlled by two co-dominant genes giving rise to the phenotypes Fy (a+b+) and Fy (a–b+). The Fy (a–b–) phenotype is characteristic of Negroes.

(9) DIEGO SYSTEM: The antigen Di^a and Di^b are determined by two allelic genes. Di^a is only seen in Mongoloids (Chinese, Japanese, and American Indians).

(10) LUTHERAN SYSTEM: The major antigens are Lu^a and Lu^b. The phenotypes are : Lu (a+b–), Lu (a–b+), Lu (a+b+). Lu (a–b–) is very rare.

(11) LEWIS SYSTEM: It is closely related to the ABO system, although the two allelic genes, Le and le are inherited independently of the ABO, Hh and Se se genes. The phenotypes are : Le (a+b) or, L_e^a, Le (a–b–), and Le (a–b+) or L_e^b.

(12) THE XG SYSTEM: The antigen X^a_g produced by the gene existing on X-linked locus gives rise to two phenotypes Xg (a+) and Xg (a–). It can be applied only when the children involved are female.

(13) PRIVATE ANTIGENS: The 'private' antigens include Levay, W^a_r, Bca, By, Rm, etc.

(14) OTHER BLOOD GROUP SYSTEMS: Some of these high-frequency antigens are: V_c^a (Vel), G_c^A (Gerbich), S_d^a (Sid), Y_k^a (York), Y_t^a (Cartwright), C^a_o (Colton), C_h^a (Chidd), R_g^a (Rodgers), D_o^a, D_o^b, Kamhuber, etc.

The agglutinogens in the stain are retained for a longer time, even though the red cells are destroyed. The absorption inhibition technique and absorption-elution method are employed for the grouping of such stains. By using mixed agglutination technique, stains on clothes or fibres can be grouped in about three year old stains.

Group Specific Substances: The agglutinogens of the ABO system are also present in body tissues. In the tissues they appear in a lipoidal form. In about 80 percent of the people they appear in a water-soluble form, and can be demonstrated in all the body fluids except the cerebrospinal fluid. They are not found in nerve tissue, epithelium, skin appendages, bone and cartilage. Persons who possess only the lipoidal form are known as 'non-secretors', while those who possess a water-soluble form are known as 'secretors'. The capacity of secreting these antigens in body fluids is controlled by a pair of allelic genes Se and se, the former being dominant over the latter. The individuals with genotype Se Se, and Se, se are secretor, and those with the genotype se se are non-secretors. Secretors possess H antigen on their red cells irrespective of their blood group of the ABO system. However, the amount of H antigen is the highest on the red cells of O group persons. The ability to secrete agglutinogens into the body fluids remains constant throughout and is transmitted as a simple Mendelian dominant. The agglutinins, a and b are also present in the body fluids. M and N agglutinogens are widely distributed in the body tissues in a relatively water-soluble form. The group specific substances in dried stains can be identified by absorption technique. The Rh agglutinogens are widely distributed in the body tissues but are not found in the body fluids, except the amniotic fluid.

Grouping a Blood Stain: About 150 mg. of blood-stained material or about 75 mg. of dried blood and the control free from stain should be available for grouping test. The agglutinogenic specificity of blood stains is retained, even though the red cells are not intact, if the stains are properly preserved. ABO retain their agglutinogenic specificity indefinitely. MNS and Rh factors lose their specificity within 3 to 5 weeks. The agglutinins present in the serum lose their specificity in the stains in a short duration. Most of the enzymes in the stains lose their specificities within 3 to 5 weeks.

Blood Groups and Heredity: Brenstein postulated the mode of inheritance of ABO system. The ABO, MN and Rh factors are inherited according to Mendelian principles. The rules of inheritance of ABO system are : (1) Agglutinogen A or B cannot appear in the child unless it is present in one or both parents. (2) Agglutinogen A_1 or A_2 cannot appear in the blood of the child unless it is present in one or both parents. (3) The combination of A_1 B parent with A_2 child, and vice versa cannot occur. Conversely, the combination of A_2B parent with A_1 child and vice versa cannot occur. (4) An O parent cannot have an AB child and an AB parent cannot have O child. (5) Parents of AO and AO genotype may have a OO child. (6) Parents of AA or AO genotype may have a A child.

The rules of inheritance for MN system are: (1) Agglutinogens M and N, cannot appear in the blood of a child unless present in one or both parents. (2) A type M parent cannot produce a type N child and conversely an N parent cannot produce M child. (3) In matings where both parents are homozygous type M or N, the children are always of the same type as the parents. (4) In matings where one parent is type M and other type N, all children are type MN. (5) In matings where one parent is homozygous (M or N), and the other heterozygous (MN), the children are of parental types in 50 to 50 ratio. (6) In matings where the parents are both MN, children of all three types are possible.

Rules of inheritance of Rh groups are: (1) Rh negative parents cannot produce an Rh positive child. (2) Rh positive and mixed parents can have Rh positive and Rh negative children.

BLOOD GROUPS IN TISSUES

(1) BONE: The determination of blood groups from bone tissue is more difficult than from other body tissues. Bones not having extractable plasma proteins, such as burnt bones and bones after 5 to 10 years following death give negative serological results. Carbohydrates, glycolipids and glycoproteins can be extracted as blood group substances from the bone

Table (20–3). Inheritance of blood groups

Phenotypes of parents	Phenotypes of Children	
	Possible	Impossible
OxO	O	A B, AB
OxA	O, A	B, AB
OxB	O, B	A, AB
OxAB	A, B	O, AB
AxA	O, A	B, AB
AxB	O, A, B, AB	None
AxAB	A, B, AB	O
BxB	O, B	A, AB
BxAB	A, B, AB	O
ABxAB	A, B, AB	O
MxM	M	N, MN
MxMN	M, MN	N
MxN	MN	M,N
MNxMN	M,N,MN	None
MNxN	N, MN	M
NxN	N	M, MN

Table (20–4) Blood group individuality.

Group of Parents	Group of children	
	Possible	Not possible
MxM	M	N,MN
MxMN	M,MN	N
MxN	MN	M,N
NxN	N	M,MN
NxMN	N,MN	M
MNxMN	M,N,MN	None

Table (20–5) Blood group individuality.

Blood groups	Numbers of genotypes	Rarest genotypes	Commonest genotypes
ABO	10	A_2A_2	O
MNS	9	NS	MNSs
P	2	P(-)	P(+)
Lutheran	3	Lu(a+b-)	Lu (a-b+)
Kell	7	Ka Ka	Kb Kb
Lewis	3	Le (a-b-)	Le (a-b+)
Duffy	4	Fy (A+b-)	Fy (a+b+)
Kidd	3	Jk (a-b+)	Jk (a+b+)
Rh	20	cc dd Ee or Cc dd EE	Cc Dd ee & Cc Dd ee

marrow. With fresh bone marrow and spongy bone, blood groups can be determined with a relatively high accuracy. Bone samples should be collected from the regions rich in red bone marrow, i.e., the proximal epiphysis of humerus and femur. In compact bone, blood group substances are thought to originate not only from bone cells but also from red cells in vascular systems. Mainly ABH blood groups are detected in the bone, but with compact bone, groups A or B are frequently misjudged as AB. MN, Gm, PGM, 6-PGD and esterase D (EsD) have also been detected.

(2) DENTAL TISSUE: Absorption-elution technique is preferred for blood grouping of dental tissues including dentine, cementum and dental pulp. Enamel contains only traces of blood group substances and grouping is very difficult. Blood grouping of a denture and dental calculus is possible if the denture has been used for a long period of time due to the accumulation of saliva. Cementum gives a weak reaction. Results are most accurate with dental pulp. Blood grouping of old teeth is possible if they are dry and not infected with bacteria. Heating at 200°C and over, destroys groups. Apart from ABO, PGM, AK, ADA, and 6–PGD can be identified from the dental pulp.

(3) HAIR: With absorption-elution technique, blood groups can be determined by a single hair shaft about six cm. in length. Blood grouping is practicable with scalp hair from foetuses and newborn infants and also with grey scalp hair. If hair is heated at 250°, it is impossible to detect blood groups. Hair left in water or soil for up to six months give good results. G6Pd, PGM, esterase D, 6 phosphoglucomate dehydrogenase, glyoxalase and a-L fucosidase (FUC) types have been detected from hair roots with sheath cells.

(4) NAILS: Three to six mg. is adequate to detect ABO groups. The human nails contain mainly ABH blood group antigens. MN blood groups have been detected in some cases. Marshall (1980) reported that proteins of human nail show a genetic variation with regard to both low-sulphur and high-sulphur protein fractions, which could serve as biochemical markers of individuality.

(5) SOFT TISSUES: The mixed agglutination technique (the mixed agglutination reaction, MCAR) is useful for detecting ABH antigens on tissue cell surfaces in all kinds of soft tissues. This technique is suitable for the direct determination of blood groups on cell fragments adhering to weapons, bullets and clothing. Decomposed muscle acquires blood group antigens different from native one, and also many bacteria have blood group antigens similar to human ABH antigens.

Medico-legal Application of Blood Groups: **(1) Disputed Paternity:** The question of disputed paternity arises in the Court in the following conditions. (1) When a child is born in lawful marriage, but the husband denies that he is the father of the child. (2) When a child is born out of lawful marriage, and the mother accuses a certain man of being the father of the child, while the man denies the accusation. (3) When a woman pretends pregnancy and delivery and obtains a child claiming it as her own, in order to obtain a share in her husband's property. (4) In suits for nullity of marriage.

Many cases can be solved by means of the blood groups of the parent and the child. In the case of the adults, 5 ml. of venous blood is taken and placed in plain tube. Neither party should have had a blood transfusion within three months, before taking the sample. The infants should preferably be at least six months of age, but not less than two months before testing is performed. One ml. of blood should be obtained by a heel or earprick, or venepuncture into a plain tube. The testing of the mother, child and putative father, i.e., the man whose potential paternity is under investigation, should be done in the same laboratory, by the same person, on the same day, and using the same batch of reagents and antisera.

However, tests have their limitations. They may exclude a certain person as the possible father of the child, but they cannot definitely establish paternity. They can only indicate its possibilities. The exclusion of putative father is based upon the principle that a specific agglutinogen cannot appear in a child unless it was present in one of its parents, e.g. if the agglutinogen A is present in a child but not in its mother. If two men are alleged to be the fathers of the child, and if one of them shows the agglutinogen A in his blood and the other does not, the person possessing agglutinogen A, must be the father. If both men have agglutinogen A, no positive opinion regarding the paternity can be given. The blood groups in current use in the investigation of cases of doubtful paternity are : ABO, MNS, Rh, Kell, Lutheran, Duffy and Kidd. The P system and the Lewis system have been well studied but are not used because of their complexity or instability.

(II) White Cell Antigens: The Human Leucocyte Antigen (HLA) System consists of protein substances on the surface of a wide variety of tissues and

organs, on tumours, white cells and platelets. They are reported to be present on spermatozoa, but not on ovum, nor on the trophoblast. They are present on the placenta at term and in foetal tissue at six weeks. They are found both on lymphocytes and granulocytes. HLA-Dr antigen is present on B lymphoblastoid cell lines, monocytes and macrophages. The major human leucocyte antigens HLA–A, B, C, D, and DR are determined by a single chromosomal segment, the 'major histocompatibility complex' (MHC), which is situated on the short arm of human chromosome 6. In white cells 68 antigenic factors are present. Each parent contributes to a child one of the two chromosomes containing the HLA region. The presently known seven multiple loci with about 125 multiple allelism, and with a very high degree of polymorphism, provide a very good discriminating capacity. Only fresh blood samples are examined in solving the problems relating to parentage. In tissue grafts, the better HLA match between subjects, the better the chances of graft's survival. The presence of these antigens may be detected by using the white cells of the blood and a suitable antiserum that either agglutinates the white cells or damages them so that they are stainable with suitable dyes that do not affect the damaged cells. Other components of this system may be detected by observing the characteristic swelling reaction of lymphocytes that do not contain the antigen, when they are placed in contact with lymphocytes that do contain it.

(III) SERUM PROTEIN POLYMORPHISM: The serum protein groups which are subject to genetic variation include the Gm and Inv types of gamma globulin, the group specific components (Gc types), the haptoglobin (Hp) types, the transferrin types (Tfc), cerulospasmins and third complement component (C_3) and several lipoprotein systems. They are usually demonstrated by electrophoresis.

(1) SERUM HAPTOGLOBINS: Haptoglobins are haemoglobin-binding proteins found in human serum. Three genotypes are present Hp_{1-1}, Hp_{2-2} and Hp_{2-1}. The pattern of inheritance is identical to that of the MN groups.

(2) GC GROUPS: Inheritance is controlled by two alleles Gc 1 and Gc 2 giving the three phenotypes Gc 1–1, Gc 2–2 and Gc 2–1.

(3) AG GROUPS: This system of serum antigens 's complex but two genes, Ag(x) and Ag(y) appear to be allelic. The possible phenotypes are Ag(x–y–), Ag (x–y+), Ag(x+y+). These factors are present at birth and not transmitted across the placenta.

(4) GM BLOOD SERUM POLYMORPHISM: In the newborn, the gamma globulin present is not its own, but has been transmitted from the mother across the placenta. As such, Gm and Inv. studies are useful in cases of mix up or exchange of babies in hospitals. The child's own gamma globulin begins to form from third month with disappearance of the Gm transferred from the mother. There are five types of immunoglobulins, IgG, IgM, IgA, IgD, and IgE which form a group of serum proteins to which all antibodies belong. Normal serum contains eight types of IgG molecules. Various Gm antigens designated Gm 1 to Gm 25 are so far determined by various allelic genes. IgG crosses placenta.

(5) KM SYSTEM: At the Km locus, there appear to be genes giving rise to four allotypes known as Km 1, Km 2, Km 3, and Km Blank.

(6) SERUM LIOPROTEINS: The Lp system has at present three phenotyes Lp (a+x–), Lp (a+x+) and Lp (a–x+). The Xm antigens are also serum lipoproteins.

(7) ABNORMAL HAEMOGLOBINS: The abnormal haemoglobins are under direct genetic control. The specific mutation is known for -more than 30 Hb variants. The abnormal haemoglobins C, D, E, G,H, I, J, K, M, S are designated in the order of their discovery. Haemoglobin C and E are associated with thalassaemia.

(IV) RED CELL ENZYME POLYMORPHISMS: A large number of enzymes, which catalyse the various vital biochemical reactions are also present in the blood, mainly in the plasma. Phosphoglucomutase (PGM) and adenylatekinase (AK) are of established value in paternity testing. Adenosine deaminase (ADA), red cell acid phosphatase (EAP), serum cholinesterase (SCE), 6–phosphogluconate dehydrogenase (G-6PD), glutamate pyruvate transaminase (GPT), esterase D (Es D), and glyoxalase I (GLO), may also be used. They are usually demonstrated by starch gel electrophoresis technique. These enzymes do not persist for more than one month in stained material.

Paternity can be excluded by ABO grouping alone in about 18%, and if MNSs system is added in about 60%. Red cell antigens and serum proteins exclude 82%. If red cell enzymes are included, exclusion will be 94%. By testing a number of HLA systems, the exclusion is 98.5%

DNA fingerprinting will positively fix or exclude paternity.

(2) Disputed Maternity: When the same child is claimed by two women, or when two children are interchanged either by accident or by design in maternity home or hospital, blood grouping tests are

helpful.

(3) Crimes: Blood stains may be found on clothing and person of suspect. If the accused alleges that the stain is of his own blood, it will have similar blood group systems and haptoglobins. If the victim has similar characters, the test is not conclusive. If there is difference in blood group of the stain and the accused's blood, then the stain is of some other person's blood. If the characteristics of the victim's blood coincide with those of the stain, an association is established between the suspect and the victim. Blood stains may be present at the scene of house breaking, e.g. on a broken window, if the culprit has cut himself. If the character of these stains are similar to that of blood of the suspect, it establishes association. Blood stains may be present under the fingernails of assailant in a case of throttling. If there has been a struggle, blood stains derived from the accused may be found under the fingernails of the victim due to scratching. Vehicles which have caused injury can be identified when they show blood resembling that of the victim.

Blood does not adhere readily to swiftly moving metallic objects, e.g. it is difficult or impossible to detect blood on a bullet which has passed through a body; sharp knives which have made a deep gash in a body may show little or no evidence of blood. In every case of murder a sample of blood (preferably from the heart) for identification should be placed in a container and labelled.

Stains on clothes due to crushing of bugs, fleas, louse, mosquitoes, etc., are common. These stains are small in size and sharply angular in outline and are usually found on the inside of the garment. If the insects are crushed, fragments of the hair of scales of the insect and eggs may be found on microscopic examination.

(4) Stains due to Body Fluids: The blood group agglutinogens can be demonstrated in stains on clothes due to semen, sweat, saliva, nasal secretion, urine or faeces in persons who are "secretors". This may be a corroborative evidence of the accused.

(5) Identity: The specificity of various blood group combinations is like that of the fingerprints. When an individual has some rare blood group, he can be identified with certainty. But when they are of common type, they are not of use.

(6) Cause of Death: In certain cases, cause of death can be established, e.g., incompatible blood transfusion. Poisons can be detected in the blood.

HAZARDS OF BLOOD TRANSFUSION: Some type of reaction will occur in about one to 2% of patients who receive blood transfusion. The antigens of the ABO and Rh systems commonly produce transfusion reactions. In the case of other antigens a number of incompatible transfusions may be required to stimulate sufficient antibodies and cause a reaction. (A) IMMUNOLOGICAL REACTIONS: (1) Intravascular haemolysis. (2) Extravascular haemolysis. (3) Sensitivity to white blood cells, platelets and plasma components. (B) NON-IMMUNOLOGICAL REACTIONS: (1) Circulatory overload. (2) Coagulation defects. (3) Hyperkalaemia. (4) Citrate toxicity. (5) Infections and transmission of syphilis, hepatitis, toxoplasmosis, AIDS, etc. (6) Air embolism. (7) Hypothermia. (8) Rigors.

The commonest mistakes in blood transfusions are: (1) clerical error, (2) confusing terms, such as "group A serum" instead of "anti-B serum", (3) failure of the staff to check the reference on the bottle against the actual laboratory report on compatibility, (4) the presence of similarly named patients in the ward.

Samples to be preserved are: (1) Sample of blood transfused. (2) Sample of blood of the receipient before and after transfusion. At autopsy, (1) kidneys (2) Blood (3) Urine.

After infection with HIV, blood becomes positive after 2 to 18 months. AIDS is usually communicated by sexual intercourse or from blood transfusion. According to guidelines laid by the Government of India, the status of HIV should not be disclosed to blood donor. The intention is to spare him of the agony of knowing the helplessness of his situation. If the blood drawn is positive, it should be discarded. Once blood sample is drawn, the register of patient-identities should be kept quite separate and samples identified only with a code number. If the donor wants to know the result of HIV test, he should be referred to an accessible HIV testing centre where supplemental tests with counselling will be offered to him.

The Centre for Disease Control (CDC), estimates that 5.5% of all HIV positive persons are employed in the health care field. According to the guidelines issued by CDC, with the exception of health care workers and personal service workers who use instruments that pierce the skin, no testing or restriction is indicated for workers known to be infected with HIV but otherwise able to perform their jobs. A person testing positive for HIV cannot be removed from service, if he is physically fit to discharge his duties.

If a person suffering from AIDS, knowingly marries or has sexual intercourse with a normal person and

thereby transmits the infection to other person, he would be guilty of offences under S.269 and 270, I.P.C.

INVESTIGATIONS: (1) HAEMATO-LOGICAL EXAMINATION: In case of intravascular haemolysis, the serum of a post-transfusion sample of blood will show the presence of haemoglobin and methaemalbumin. Haptoglobins will be reduced. In extravascular haemolysis, there is an increase in the quantity of unconjugated bilirubin.

(2) URINE: In intravascular haemolysis, haemoglobin will be found in urine. Urobilin, urobilinogen, and red cell casts may be found.

(3) SEROLOGICAL EXAMINATION: A two percent red cell suspension in saline of the patient's blood may show agglutinates. Coomb's test is positive. Ig A specific antibodies will be found in the patient's blood in sensitivity reaction to donor leucocytes, platelets and plasma factors. There may be eosinophilia.

(4) BACTERIOLOGICAL EXAMINATION: Residual donor blood may show microorganisms, which can be confirmed by culture.

(5) AUTOPSY: In acute intravascular haemolytic reactions, haemoglobinuric nephrosis is seen. The tubules will show acute necrosis and casts of haemoglobin. Lungs will be oedematous in cases of circulatory overload. Air in the right ventricle indicates death due to air embolism.

SALIVA: It contains enzymes like ptyalin, glucose 6 phosphate dehydrogenase, various proteins, lipids, chlorides, thiocyanate ions, etc. The stains are identified from the presence of amylase and buccal epithelial cells. Amylase activity can be measured by the starch-iodine test or Phadebas test. ABO grouping and species origin can be carried out.

FAECES: The stains can be identified from odour, presence of undigested muscle and vegetable fibres and stercobilin.

URINE: The stains can be identified from the presence of urea, uric acid and creatinine.

VAGINAL SECRETION: It consists of white coagulated material consisting of shed vaginal epithelium and Doderlein's bacilli.

DNA FINGERPRINTING

DNA fingerprinting (DNA typing, DNA identification, or genetic typing) is a technique involving chemically dividing the DNA into fragments which form a unique pattern and then matching that "identity profile" with the pattern obtained from similarly testing a suspect's blood specimen. If the two patterns match, the possibility of error, i.e. the chance that they do not belong to the same individual may be less than one in 30 billion. Dr. Alec Jeffreys in 1985, developed DNA fingerprinting.

Human body consists of about six thousand billion cells which constitute tissue and organ systems. Every living cell has genetic material contained in units called chromosomes, which are located in nucleus. DNA is present only in nucleated cells. Each human somatic cell has 23 pairs of chromosomes of which 23 are derived from biological father, and 23 from the mother, due to fertilisation of ovum with sperm. Genes are arranged along the length of each chromosome, which are responsible for various functions of the body. Each gene carries instructions for the production of a particular protein which performs a particular function. Genes are also responsible for transmission of heredity. The genes are made up of chemical molecules called deoxyribonucleic acid (DNA). The human genome contains about 6×10^9 DNA molecules per diploid genome.

The core of the chromosome is a very long and extremely thin thread of DNA. A single human chromosome is about 1/12,500 cm. long. The DNA molecule in this chromosome is about 2.5 cm. in length, compacted into the chromosome by successive coiling.

The total DNA in a cell is about 180 cm. in length. Each chromosome consists of two long linear DNA molecules, the polymers being hydrogen bonded via specific nucleotide pairing and coiled as a double helix which is spiral in nature, and looks like a spiral staircase. The helix is structurally stabilised by nuclear proteins called histones, the complex of DNA and histones being referred to as chromatin. Chromatin may be condensed to varying degree of compactness and in its most compact form is seen microscopically as chromosomes at the metaphase stage of each cell cycle. The chromosomes are continuous strands of DNA ranging from 50 to 500 million molecules per chromosome, encoded in this.

Each nucleotide is composed of phosphate, deoxyribose sugar, and organic nitrogenous base. The bases are adenine (A), guanine (G), cystosine (C), and thymine (T). The bases of one strand are connected to the bases of the other strand by hydrogen bonds, while adjacent nucleotides are linked with each other by covalent bonds. Adenine combines only with thymine and guanine combines only with cytosine. The DNA molecule resembles a twisted rope ladder with four kinds of stair-steps, e.g., A-T, T-A, C-G, or G-C. There are three hydrogen bonds between G and C, and 2 bonds between A and T.

A single DNA molecule consists of 50 to 500 million base pairs. The two strands of DNA helix run in opposite direction. The base sequence of one strand is always complementary to the sequence on the other. Each segment of DNA in a chromosome which codes for a particular protein is called a gene. In the human genome there are about 10,000 genes, accounting for about 5% of the entire cellular DNA. In between the active base pairs which code for a particular protein, there are large number of redundant/inactive base pairs forming 95% of DNA, which is considered as "junk DNA'. In junk DNA short sequences of base, repeat themselves over again like a stutter (repetitive DNA), e.g. GCTA, GCTA, GATA, GATA, etc. The regions containing repetitive DNA demonstrating hypervariability from person to person are called "satallite DNA", which shows an extremely high degree of variability, and these variants are called "variable number tandem repeats" (VNTR) or "minisatellites". There are more than 1500 VNTR's in the human genome. Selected regions of VNTR are broken into fragments using special enzymes (restriction endonucleases), which are individualistic in nature and establish 100% identity.

DNA is a robust molecule which can tolerate remarkable range of temperature, pH and other factors. DNA mixed with detergents, oil, gasoline and other adulterants does not alter its typing characteristics.

There are two methods of DNA analysis in common use. (1) RFLP (restriction fragment length polymorphism). (2) PCR (polymerase chain reaction).

(1) RFLP Method: DNA can be extracted from any body fluid or tissue in which nucleated cells are present. All the samples should be frozen at –20°C before use. The samples (blood, bone marrow, semen, hair roots, tooth pulp, tissue from any organ, or skin) are usually examined. The separation of DNA involves (1) disruption of cells and fractionation of cellular organelles, (2) dissociation of DNA from proteins by the use of salt solution or detergent, (3) addition of an extractant to phase-separate the bulk of the protein from the DNA, (4) use of enzymes or differential precipitation to remove the RNA and polysaccharides. The isolated DNA is quantitated by ultraviolet spectrophotometry. DNA is completely digested with restriction enzymes called restriction endonucleases. These enzymes recognise the specific sequence in the double strand DNA and cut the DNA at this site into various fragments, called as rectriction fragment length polymorphism (RFLP). RFLP's are produced due to variations in human DNA. These variations in restriction fragment lengths is due to presence of variable number of tandem repeats (VNTR). Most of repeated DNA are arranged as short sequences repeatedly contiguously in tandem, hence called VNTR. Several restriction endonucleases are in use, e.g., Eco-R-1, PsT-1, Hin F-I (obtained from E.coli), Sau 3A-1 (obtained from Staphylococcus aureus), Hae-III (obtained from Haemophilus influenzae). PsT-1 (a six-base cutter) is commonly used, which recognises the sequence CTG, CAG.

Digested DNA is run on agarose gel electrophoresis. The different restriction fragments are separated varying in length between 0.5 to 25 kb, which varies from one individual to another. The smaller fragments move much faster through the gel than the larger one. The gel is later stained with ethedium bromide for 40 minutes which tightly binds to DNA and fluoresces under UV light.

From the agarose gel, DNA is transferred to nylon membrane using capillary transfer technique of Southern. The result is a mirror-image replica of fragment distribution. Vacuum blotting of transfer is used more commonly as it is less time consuming. DNA is then fixed by heat at 80°C or cross-linked by the cation of UV irradiation.

Next hybridisation is done which is the pairing of two complementary single strands of DNA to form double stranded DNA. It involves the addition of a probe to the nylon membrane. A probe is a single-stranded recombinant DNA segment, or synthetic DNA, which is designed to go to a particular predetermined locus on a particular chromosome. It is usually tagged with a radioactive marker, such as P_{32}. The probe scans all the DNA fragments and wherever it encounters its complementary sequence, it will hybridise with it, thereby making the fragment radioactive. Usually four probes are used one at a time, due to which four different regions of the DNA would be analysed.

In the Centre for DNA Fingerprinting and Diagnostics (CDFD), Nacharam, Hyderabad-76, A.P. India, BKm probe is used, which is a multilocus probe isolated from the female banded krait, as a minor satellite DNA. More than 36 hypervariable VNTR are detected by BKm.

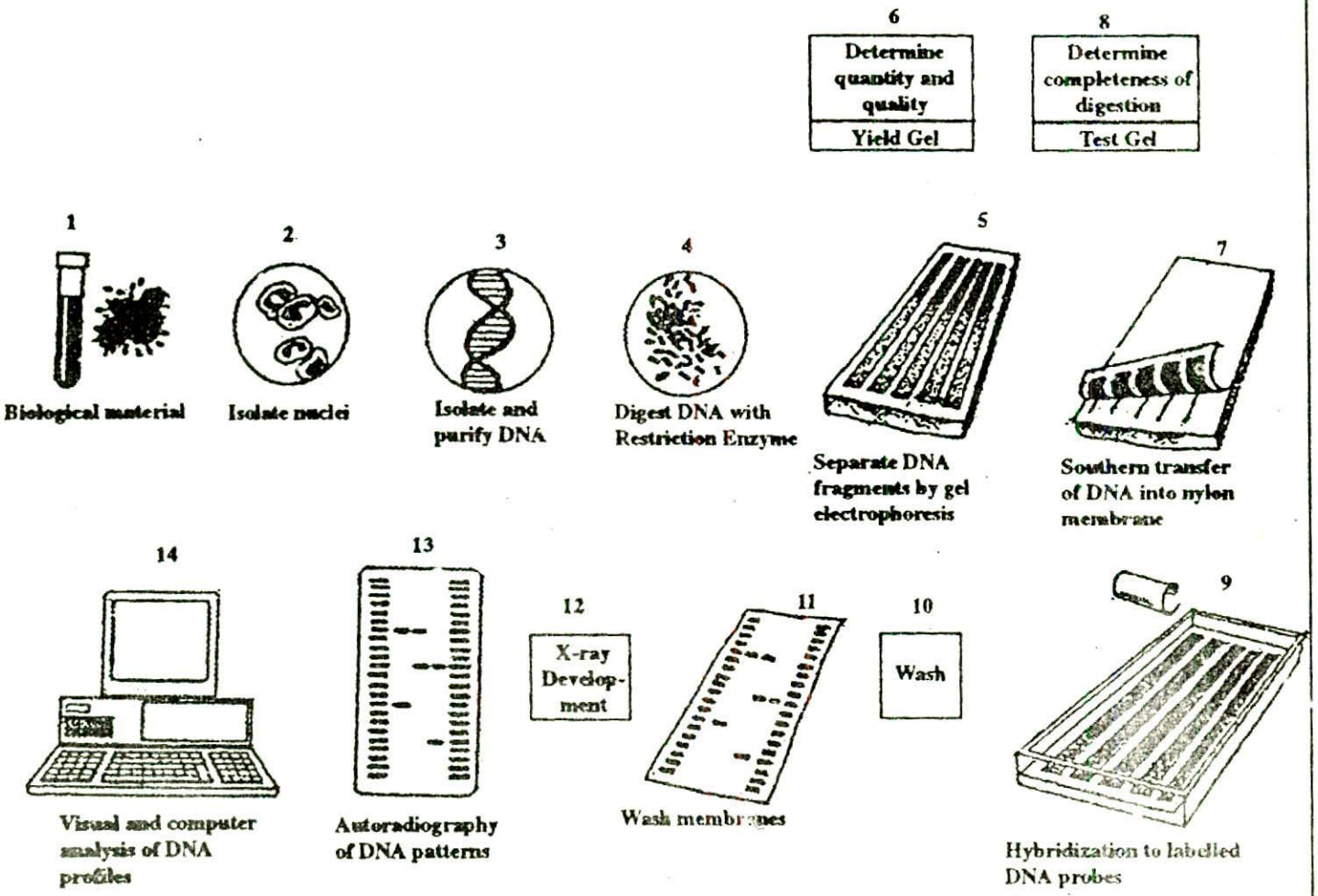

Fig. (20–4). Procedure of DNA fingerprinting

The label incorporated into the probe permits to localise these sites. After hybridisation, the membranes are washed with 0.05% SDS which removes loosely bound probe. The membrane is then wrapped in the saran wrap and placed in the X-ray cassette holder along with X-ray film, and kept at 80°C. Exposure time depends upon the specific activity of the probe ranging from few hours to days (up to 10 days). X-ray films are then developed and fixed in the respective reagents and finally wahsed in water and dried. This autoradiograph is a permanent record, in which grey to black bands are seen where the radioactive probes had hybridised to fragmets bearing complementary sequences. The series of bands seen on the film is called an "autorod", which represents the DNA fingerprint of that individual from whom the DNA had been obtained. The pattern of bands is unique to each individual.

(2) POLYMERASE CHAIN REACTION (PCR): This technique is used when a very small amount of DNA or a partially degraded biological material is available. A small amount of DNA is amplified more than a million-fold using thermal TAQ polymerase. It is particularly useful for diagnostic purposes. DNA is isolated and split apart, by heating the sample to more than 94°C. Later, the temperature is lowered, during which small segments of DNA (primers) bind specifically to the DNA which has the polymorphic regions of interest. The primers act as the starting point for the DNA duplication process. The temperature is again raised to 72°C which causes DNA polymerase (TAQ polymerase) to extend the primers and copy the two separated strands of DNA. The TAQ polymerase utilises nucleotides by inserting them opposite the appropriate base, thereby ensuring the integrity of DNA's base pair complementarity. The cycle is repeated for 30 times or more which synthesises more than a million copies of DNA. Detection of amplification products for VNTR polymorphism is done by electrophoresis, followed by visualisation by fluorescent detection or silver staining.

PCR technique can analyse 36 samples at a time. It is sensetive and quicker method but less specific than RELP.

The various techniques in use at present are: (1) Restriction fragment length polymorphism (RFLP) using (a) Multi-locus probes (MLPs) (b) Single-locus probes (SLPs) (c) Variable number tandem repeat (VNTR) sequences.
(2) Polymerase chain reaction (PCR).

In case of MLPs the probe used detects variations at several genetic regions simultaneously. The band pattern produced on X-ray plate produces a strip of 30 to 40 dark bands.

SLPs analyse only single hypervariable location in human DNA. These play a very major role in forensic practice as they have far greater detection sensitivity than the MLPs. Each SLP detects just two bands (one maternal and one paternal). The sensitivity is such that a single hair root can be identified. Results can also be obtained from degraded DNA (often found in forensic samples) as SLP detects the remaining, non-degraded alleles among the DNA fragments. As they detect only two bands/SLP, using single SLP reduces the probability to 1/10000 population as compared to 1 in 10^{12} MLP. Using multiple SLPs is therefore the practice now-a-days. SLPs are human specific, MLPs detect DNA fingerprint in all vertebrates. 80% of forensic work depends on SLPs.

Multilocus or single locus probes are either cloned probes or synthetic oligonucleotides.

VNTR: This method uses another set of probes which detect specific variable number tandem repeats of a sequence. These also remember the minisatellite in that they consist of a repeated sequence with the number of copies of the sequence varying from one person to the other. However, where there are usually many minisatellites of a given type in a genome, there is only one VNTR of each type. These probes therefore produce simpler banding patterns. Several VNTR probes are used, each of which recognises one VNTR site, to characterise a DNA sample. After the frequencies of the various bands produced by each VNTR probe have been established for each ethnic group, these can be used to calculate the probability of any particular combination of patterns occurring in each individual. The basic method is similar to the RFLP method.

The main disadvantages of RFLP method are: (1) The samples have to be in good condition to be analysed. (2) Fragments isolated/identified by this method are in the ranges of 2 to 20 Kbps.

The advantages of PCR over the RFLP are: (1) It is relatively simple and easily carried out in the laboratory. (2) The results are obtained within few days. (3) It permits analysis of extremely tiny

amounts of DNA. The disadvantages are: (1) It is susceptible to contamination. (2) Most PCR loci have fewer alleles than the VNTR areas utilised in RFLP. (3) Some of the PCR loci are functional genes.

Most of the forensic work using SLPs, MLPs are the method of choice if the sample being tested is in a good amount and condition. A single MLP, by detecting numerous hypervariable loci simultaneously, yields a large amount of information in less time. Band pattern of SLP is simple (only two bands per SLP), hence easy to analyse; amount of sample needed is very small as detection is highly sensitive. But to increase specificity a large number of SLPs are to be used. Therefore time taken is very much.

STR (short tandem repeats): STR is a PCR technique that may replace RFLP and PCR methods in forensic labs. In this system, the repeat unit is much smaller than VNTR loci, being normally 2 to 6 base pairs. STR loci occur throughout the genome at a frequency of about 1 STR for every 3 to 5 lakhs base pairs. Most STR loci have only 6 to 12 alleles, but there are a large number of such systems that can be exploited for identification purposes, which can produce a high power of discrimination in combination.

This technique is more rapid and can be done in 2 to 3 days. It can be performed on small quantities of DNA. It is possible to run the products from several STR loci simultaneously on one gel as long as the fragment sizes do not overlap.

STR analyses are performed by: (1) isolating the DNA, (2) replicating the STR fragments by PCR, (3) performing gel electrophoresis, and (4) identifying the fragments using stains, chemiluminescence or laser techniques.

Even though two persons share an overwhelming proportion of their DNA, there are still enough differences that no two persons are genetically alike, unless they are identical twins.

SAMPLES (BIOLOGICAL) ENCOUNTERED IN FORENSIC PRACTICE

(1) BLOOD : Stains on cloth/wood/metal/plastic/ floor titles/wall paper/news paper/food. (2) SEMEN: Stains on cloth/paper/furniture/floor tiles, anal/vaginal/ buccal/penile swabs, vaginal aspirates, fur/matted hair (pubic/axillary/scalp, etc.) (3) HAIR: Head/body/pubic/ scalp. (4) TISSUE: Bonemarrow/muscle/spleen/ fingernail scrapings. (5) MOUTH: Swabs. (6) FOETUS: Muscle biopsy/chorionic villous samples. (7) SALIVA STAINS: Cigarette butts/envelopes/also nasal mucus stains.

COLLECTION AND FORWARDING OF FORENSIC SAMPLES FOR DNA FINGERPRINTING: The samples collected at the scene of crime along with the control samples should be sent to the DNA typing laboratory within the minimum period of time.

(1) LIQUID BLOOD: In cases of paternity, maternity disputes, biological relationships, etc., or as control samples in criminal cases, 2 to 5 ml. of i.v. drawn blood should be collected in sterile leakproof preferably screw-capped tubes containing heparin or EDTA as anticoagulant. The sample should be mixed thoroughly but slowly, and placed in a container containing ice, or a thermos flask. The sample tube should be sealed and labelled containing the names of the source of the blood sample, the name of the doctor collecting the blood sample, time and date of collection, name of forwarding authority, etc. The samples should reach the laboratory within 24 to 48 hours. If the person is suffering from visible genetic disorder, it should be mentioned in the forwarding letter of advice.

In cases of crimes, a blood clot can be transferred by using a clean cotton cloth. Dried blood stains on weapons, garments, etc. can be left intact and entire object submitted. Dried blood stains on large immovable articles can be scraped into a clean piece of paper, or the stain can be lifted from the surface using adhesive tape, or sample can be eluted by rubbing the stained area with cotton swab moistened with distilled water.

(2) SEMEN, VAGINAL SWABS: Sterile cotton ear buds can be used as swabs. After collection, these swabs should be completely air-dried, and placed in a dry sterile tube, sealed and labelled with necessary information. Other relevant information about the sample should be sent separately along with the sample. In gang rape cases, 3 to 4 vaginal swabs should be collected and sent in separate tubes. The surrounding areas of the private parts of victim should be swabbed with wet cotton swabs moistened with sterile water. These swabs should be packed in separate vials. The clothes worn by the victim at the time of the offence, should be air-dried and packed in papers. The sample should not be dried under direct sunlight or by any artificial method. If there is delay in dispatching the samples to the lab, the vaginal swabs should be stored at 4°C in a refrigerator. The cloth material should be stored at room temperature. The vaginal smeared slides should be packed individually and sent at room temperature to the lab. Dried stains on immovable articles can be collected as in the case of blood stains. The blood samples of the accused should be collected in a glass bottle as described earlier.

(3) SALIVA: Saliva in liquid state or stained area, as much as possible should be sent in dried condition.

(4) STAINS FROM SCENE OF CRIME: Stains from body fluids at the scene of crime or large objects should be swabbed with sterile cotton buds moistened with sterile water, air-dried and placed in a clean bottle and sent to lab at room temperature.

(5) BLOOD/SEMEN STAINS FROM INDIVIDUALS OR IN FIELD CONDITIONS: Blood/semen from the individuals could be collected in sterile conditions and spread on sterile bandage cloth folded several times so as to make it absorb all the body fluid. This should be air-dried in shade and placed in a clean envelope, sealed, labelled and sent to lab at room temperature.

(6) URINE: Urine about 10 ml should be freezed, or stain as available should be sent in dried condition.

(7) HAIR: Hair can be picked up by using a forceps without damaging the root.

(8) VISCERAL SAMPLES: In mutilated bodies, samples of 100 g. of muscle should be dissected using sterile instruments and placed in a sterile glass or polypropylene tube containing normal saline, as a preservative. If available, a 20% solution of dimethyl sulphoxide (DMSO) saturated with sodium chloride can be used as preservative. In cases of mass disasters, air crashes, bomb blasts, etc., where several pieces of body are found, sufficient amount of muscle may be collected individually and sent as separate exhibits. In exhumations when dry tissues are present, they should be placed in a sterile tube without preservative and sent at room temperature to the lab.

In case of foetus, the placenta should be removed and only foetus sent in normal saline or DMSO. The jar containing foetus should be placed in a thermocole box containing ice and sent to the lab.

(9) BONES, TEETH AND HAIR: Femur and humerus yield more bone marrow and are preferred. If skull containing teeth are found, molar teeth from upper and lower jaws should be detached and sent. If molar teeth are not available, other teeth may be sent. Bones should be packed in clean paper or cloth. No preservative is necessary. Teeth should be placed in a clean polythene cover. Skull containing teeth, which has been used for superimposition test is not useful for DNA analysis. As such few teeth should be extracted from upper and lower jaws before cleaning for superimposition.

Hair (preferably with roots) should be packed in a clean paper. No preservative is required.

(9) FINGERNAIL SCRAPINGS: The palm of the victim should be placed on a clean polythene sheet, and the inner portion of the fingernails scraped with a toothpick, etc. and placed in a polythene sheet.

SAMPLE PRESERVATION: Freezing is simplest procedure. For long storage –70°C up to 5 weeks; up to 5 days storage in ice. The tissue samples should be wrapped in aluminium foil, placed in plastic bags and frozen. Fixation by formaldehyde is not recommended. Dried stains should be collected in clean envelops and maintained dry or frozen.

AUTHENTICATION AND FORWARDING: Blood samples in cases of paternity disputes and in cases where they are used as control samples for identification purposes should be collected in the presence of judicial officer. The samples should be sealed, and a specimen of the seal on paper, should be sent along with the samples for verification. The identification card and the forwarding note should be filled, certified and sent to the lab along with the samples. In persons who had blood transfusion within three months preceding the date of collection, the samples are not useful.

The material can be forwarded to the lab by Executive of Judicial Magistrates, S.I. of Police or above ranks, Asst. Civil Surgeons and above rank.

Applications: (1) Murder: The blood on a weapon can be matched against the blood of the victim. Blood stains on the clothing or the person of the accused in a case of murder can be matched with the blood of the victim. Hair roots found on a weapon can be matched against the blood of the victim and accused.

(2) Sexual crimes: In sexual crimes, the seminal DNA obtained from the vaginal aspirates or swabs, or from the skin or clothing of the victim is printed and compared with the DNA prints obtained from blood samples of the suspects. If they match, the suspect is criminal, otherwise not. If a condom is recovered it should be frozen intact.

(3) PATERNITY DISPUTES: In paternity dispute cases, the blood of the child, mother and alleged father are printed for DNA. A child will have 50% DNA from mother and 50% from father. The bars in the child's code are matched first with the patterns of the mother. The remaining bars are then matched with the patterns of the father. If they correspond, he is the father. The parents should not have had a blood transfusion within three months, before taking the sample.

(4) Identification of **mutilated remains** as in cases of accidents, mass disasters, bomb blasts, burnt bodies, putrefied bodies, etc. The DNA fingerprint obtained from such remains can be

compared with previous prints if available or with that of the close blood-relatives of the deceased, which can establish links between family members.

(5) Extortion cases: Saliva samples from envelopes, face masks, nasal secretions, saliva from cigarette butts, etc.

(6) Identification of bodies in exhumation cases.

(7) For tracing pedigrees and for establishing family relationship.

(8) All cases of biological identification.

(9) To exonerate a falsely implicated person of any crime.

The test can be done even on very old stains or specimens.

CASES : (1) A Ghanian boy living in U.K. emigrated to Ghana to join his father. When he decided to return to U.K. the immigration authorities, suspecting a replacement/substitution refused entry. Conventional blood and genetic marker tests showed that the mother and the boy were related, but the possibility that the woman was the boy's aunt could not be ruled out. DNA test carried out by Jeffrey proved that the boy and the woman were in fact mother and son.

(2) Loraine Benson was murdered near Raynes Park station in London just before Chrismas'88. Several items were submitted to FSL including a man's hanky, stained with blood. The blood stain proved to be Loraine's. A nasal mucosal stain was also found on hanky which was profiled. This was not Loraine's. No semen/alien blood stains was detected. Early in February '89, a rape attempt occurred close to the murder scene. A suspect, Dumme, was arrested after a chance fingerprint was found and tallied. Police thought him to be a likely suspect. His blood sample was profiled. This matched with the profile obtained from the mucosal stain on the hanky. Dumme pleaded guilty.

(3) A rape victim thought that she had recognized the assailant from her school days. After positive identification at an identification parade, a blood sample was sent for DNA analysis and compared with DNA profile obtained from rapist's semen. The suspect was eliminated. However the DNA banding proved similar thereby suggesting that a close relative of the suspect could be the assailant. A sample from the brother of the suspect proved positive and he pleaded guilty.

This case is particularly interesting as it proved highly protective for an innocent man. Traditional blood grouping test might well have produced results quite similar to his brother. That and a positive identification based on past acquaintance could have put him in real danger of miscarriage of justice.

In Nalini Vs state of Tamilnadu, the Supreme Court upheld the admission on DNA fingerprinting evidence.

Several convictions have occurred in India, in which DNA fingerprinting has been accepted as evidence under S.45, of Indian Evidence Act.

Problems associated with profiling are:

(1) The technique is essentially an autoradiographing technique in the sense that the radioactive probes activate X-ray film. If the hybridization is imporper/ if activation is less, the band pattern might change. (2) The scientists interpretation of the band pattern might be inaccurate due to inability to visualise the patterns properly. (3) Unlike the fingerprints, which can be enlarged and shown in the Court, allowing the judges to make up their minds about points of resemblance, DNA profile are very minute. Resolution may be very faint, hence the expert's evidence is taken on trust and faith.

There are yet no proper International guidelines. Each lab has its own control/standardization methods. As the test is a fairly complicated procedure requiring stringent control measures, labs may make mistakes. Court is unlikely to understand in any detail the principles of the process. In DNA profiles/fingerprints everything is taken on trust. Expert witnesses put an interpretation upon an autoradiograph, assert odds of random matches, explain apparent non-matches.