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MATURATION OF FETAL BODY SYSTEMS

Report of a WHO Scientific Group

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WHO SCIENTIFIC GROUP ON MATURATION OF FETAL BODY SYSTEMS

Geneva, 21-27 August 1973

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MATURATION OF FETAL BODY SYSTEMS

Report of a WHO Scientific Group

A WHO Scientific Group on Maturation of Fetal Body Systems met in Geneva from 21 to 27 August 1973. The meeting was opened by Dr W. H. Chang, Assistant Director-General, on behalf of the Director-General.

1. INTRODUCTION

An infant that is mature at birth may be defined as one that shows functional competence, providing a reasonable safety margin, in the environmental and other circumstances to which it is normally exposed. For immediate survival the cardiovascular, respiratory, thermoregulatory, and endocrine systems must be competent. It is not essential that the skeletal muscles, other than those with respiratory functions, or the distance receptors, auditory or visual, should work well at birth. Immunological competence is vital, especially where passive protection is not available.

The different organ systems mature at different rates during gestation. An effective circulation must be established with placentation, but the lungs and kidneys are not needed for survival until birth, though they may be necessary for normal fetal development. In the intermediate period the fetus is not an inactive passenger *in utero*. It shows a wide variety of nervous and muscular activities, including breathing movements, that become increasingly vigorous and varied with age; for these the circulatory, metabolic, endocrine, and nervous support systems must be provided. Measurement of each of these and further study of their integrated function is required, as well as an appreciation of the determinants of growth, for a good picture of maturation. In some instances natural perturbations may be used to assess the ability of the fetus to participate in maintaining its environment and to grow in relation to functional and pathological changes. Such an opportunity is afforded by premature delivery, with reservations related to the cause of the onset of labour where they are known.

The relative inaccessibility of the fetus, which is such an important feature of its defence mechanisms, has been a major barrier to systematic scientific study. While acknowledging the considerable contributions of classical embryology, biochemistry, and physiology, it should be noted that animal studies, particularly in the last 15 years, have established a new framework of knowledge that has illuminated hitherto obscure perinatal disease. But our knowledge is still fragmentary.

The objectives of this Scientific Group were (1) to identify, in relation to selected body systems and within the special fields of expertise of the participants, some areas in which lack or disorder of maturation contributes to the excess morbidity and mortality of the human infant, and (2) to review current methods of assessing this maturation before and after birth in the human and to define the control mechanisms, maternal and fetal, where these are known. The Group then reviewed selected animal studies that have given and continue to give insight into the control and assessment of maturation, particularly when relevant studies in man are ethically or technically impossible. The Group discussed some of the difficulties that are encountered in interpreting the results of nonhuman studies in relation to the human fetus and infant. Throughout the text, areas of probable or possible future development are presented. Finally, specific recommendations are made for future studies on animals and humans.

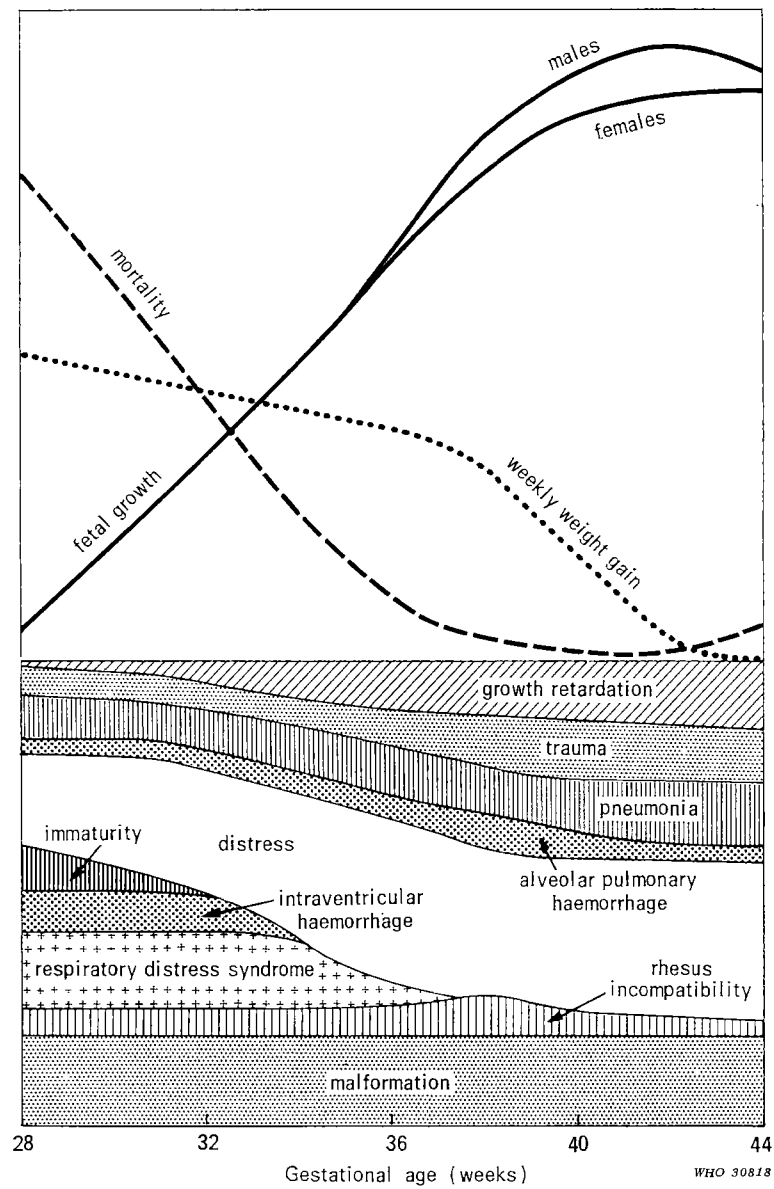
2. COMMON CLINICAL PROBLEMS

The British Perinatal Mortality Survey has provided invaluable information in connexion with causes of death. It was noted in the second report¹ that "... maturation is prominently associated with gestational age, as has been amply demonstrated by clinical as well as postmortem studies ... Since various biological functions mature at different times, the incidence of pathological conditions caused by one or another aspect of immaturity (in conjunction with external agents) varies with different gestational ages." The proportions of abnormalities found at autopsy in infants of different gestational ages are shown in the accompanying figure. Conditions associated with immaturity would be expected to be more prominent at 28 weeks than at 40 weeks. Note that intraventricular haemorrhage and the respiratory distress syndrome are the only two conditions, other than "immaturity" itself, that are restricted to infants of lower gestational ages.

It is a complex problem to decide which malfunctions are limiting to life, and to determine the causes of morbidity and mortality. The clinician faces the paradox that some infants born after short gestations of 26-28 weeks, and of accordingly low birth weight, appear to have an uncomplicated postnatal course. Others born at 36 weeks, also of appropriate weight, may have evidence of immaturity of one or more organ systems. It is now recognized that body weight is not always an appropriate guide

¹ Butler, N. R. & Alberman, E. D. (1969) *Perinatal problems. The Second Report of the 1958 British Perinatal Mortality Survey*, London, E. & S. Livingstone Ltd.

INCIDENCE OF SPECIFIC CAUSES OF DEATH AND BASIC DATA ON FETAL GROWTH
AT DIFFERENT GESTATIONAL AGES¹



¹ From: Butler, N. R. & Alberman, E. D. (1969) loc. cit.

to gestational age, or *vice versa*. While fetal growth charts depict the percentiles for weight at different gestational ages, significant departures from predicted growth are now commonly acknowledged, for example, after certain fetal insults, such as rubella, after restriction of placental blood flow in the experimental animal, or in association with a number of congenital malformations. Only recently has it been recognized that in a given infant the degree of maturation of any one organ system could be relatively accelerated or delayed.¹

The incidence of conditions that contribute to perinatal morbidity may vary widely in different parts of the world, but premature onset of labour is a major factor everywhere, with highest mortality in the more immature infants.

Maternal factors that are associated with high risk to the infant include infections, malnutrition, hypertension, abnormalities of the uterus, cephalopelvic disproportion, cord accidents, and placental abruption. Adverse fetal-maternal interactions can occur as in isoimmunization of the mother by fetal antigens.

Disorders that may have their genesis *in utero*, but express themselves in the newborn infant, include hyaline membrane disease, pulmonary haemorrhage, intraventricular haemorrhage, haemolytic disease of the newborn, and structural and functional malformations. Longer term consequences include, for example, neural and muscular disorders.

Acquired neonatal disorders are much more frequent in the prematurely born, and include infection, haemorrhage, and apnoeic spells, but at times death occurs in the absence of other detectable changes. Undersized infants have little fat, and thus are dependent on adequate calorie intake. Appropriate feeding is hard to achieve in very small infants, in part because of lack of knowledge of the best food to provide, and in part because the immature gastrointestinal tract may not absorb food adequately. Necrotizing enterocolitis is an increasing problem among babies with low birth weight who survive their initial respiratory distress and live for 2 or 3 weeks. Another major problem among these infants is persistent patency of the ductus arteriosus, occasionally leading to heart failure.

Systematic observations on prematurely born infants can define the extent of functional immaturity, the processes that "mature" faster as a result of birth, and life-limiting malfunctions. Further delineation of the chemical stages of maturation, discovery of regulators of maturation, and knowledge of the long-term effects of forcing differentiation either pharmacologically or by premature delivery should permit improved management of immature infants.

¹ Kotas, R. V. et al. (1971) Evidence for independent regulators of organ maturation in fetal rabbits, *Pediatrics*, 47, 57.

3. REGULATION OF FETAL GROWTH AND PLACENTAL COMPETENCE

The size and weight of the mature infant vary widely in most of the species studied, especially those with long gestations. Divergence in growth rates takes place mainly in the latter half of pregnancy and is influenced by different genetic and environmental factors. While there exists a general correlation between the weight of the infant and that of the placenta, significant exceptions can be found and placental weight cannot be considered a reliable indication of fetal growth. Thus, while the placental growth curve in sheep and in man flattens much before term, the development of placental structure and transfer continues along with fetal growth. Whether changes in placental function in late pregnancy become rate-limiting to fetal growth is not yet known. Observations in sheep and in man suggest that the transfer function of the placenta is sufficient to support fetal life well after term.

Regulatory factors affecting fetal growth are manifold and have a quantitatively different impact. An order of importance has been proposed¹ for the effect of these factors on the human fetus as follows: (a) intra-uterine and fetal environment, (b) maternal genotype, (c) maternal environment, (d) fetal genotype, (e) parity, (f) maternal health and nutrition, (g) sex of fetus, and (h) maternal age. Experimental studies in animals support, in general, these deductions but have also led to the recognition of some factors not yet studied in man.

Infants of low birth weight, other than immature (preterm) infants, present a common paediatric problem. Attempts made to separate them into classes, such as growth-retarded (dysmature) and small for gestational age infants, have not led to a standard nomenclature. Such aberrations in growth, almost exclusively reductions, may result from infections, teratological influences, chromosomal errors, maternal malnutrition, high altitude, and other factors.² Prominent among the factors leading to fetal growth retardation, however, are maternal pre-eclampsia and multiple births, particularly the birth of monozygous twins. In pre-eclampsia it is believed that growth retardation results from a reduction of maternal placental perfusion with subsequent reduction in the placental exchange surface. Similar mechanisms cause retardation in some of the haemoglobinopathies. On the other hand, the severe growth retardation in one of a pair of monozygous twins with the "transfusion syndrome" is attributed to haemodynamic changes in the fetal-placental vascular system.

¹ Penrose, L. S. (1961) *Genetics of growth and development of the fetus*. In: Penrose, L. S., ed., *Recent advances in human genetics*, Boston, Little Brown & Co.

² McClung, J. (1969) *Effects of high altitude on human birth*, Cambridge, Mass., Harvard University Press.

Many infants with prenatal growth restriction remain permanently growth-retarded despite adequate postnatal nutrition.

The methods used to assess fetal growth in man fall into two main categories, those that measure fetal size and those that depend on maturation of particular fetal body systems. The latter usually reflect total fetal growth, but may not do so in abnormal circumstances.

Few clinicians have confidence in their ability to determine fetal size accurately by palpation. Serial radiological studies cannot be supported because of their inaccuracy and potential fetal hazard. Measurements with ultrasound are probably the best available means of estimating fetal size and growth; sequential measurements of trunk size and skull size improve accuracy.

The value of measuring the maternal blood levels, or the urinary output, of a variety of compounds has been explored. For example, it has been known for many years that there is a statistical relationship between urinary pregnanediol excretion and fetal growth. Individually, the relationship is not sufficiently close for useful clinical purposes. Serial measurements of urinary estriol and perhaps estetrol continue to provide information of some value in high risk pregnancies. They have a reasonably close relationship with fetal growth and particularly with changes in fetal growth. The value of assay of these hormones in maternal plasma is not yet clear. Assay of human chorionic somatomammotrophin should, as far as is known, be regarded as complementary to, rather than an alternative to, measurement of estriol. The large amount of data available on changes in some enzymes has not been of significant assistance. In pre-eclampsia, serial measurement of fibrinogen degradation products may be useful.

The clinical usefulness, in the assessment of fetal growth, of determining the levels of amniotic creatinine, bilirubin, and the proportion of fat-staining cells has not been firmly established. Measurements of lecithin or lecithin-sphingomyelin ratios, perhaps together with assay of amniotic cortisol, are very useful in assessing maturation of the lungs.

Many animal species have been employed in the search for answers to the specific growth-limiting factors in fetal development. In an early experiment on crossing Shire horses and Shetland ponies¹ the size of the newborn foal was thought to be a reflection of that of the dam. The inference drawn from this study that maternal size has a large influence on fetal size—estimated by some to be responsible for 50–75% of fetal size variability—is not uniformly accepted and the mechanisms are not clear. Many are of the opinion that nutrition, in the larger sense or in the restrictive sense of uterine perfusion, limits fetal size, either by limiting

¹ Walton, A. & Hammond, J. (1938) The maternal effects on growth and conformation in Shire horse-Shetland pony crosses, *Proc. roy. Soc. B.*, **125**, 311.

fetal and placental growth, or by limiting placental growth and thereby fetal growth.

Reduction of uterine vascular perfusion has been achieved by ligation of arteries in rats and a few other species.¹ It may lead to severe fetal stunting but more quantitative measurements of the relationships of reduced flow to fetal growth restriction are needed. Similar effects may be seen in maternal treatment with vasoconstrictive drugs.

Severe growth retardation can be achieved in rhesus monkey and sheep fetuses when the size of the fetal placenta or of the placental vascular bed is experimentally restricted by ligation or microsphere embolization. It also occurs when the uterine caruncles of the sheep are severely reduced surgically before pregnancy. It is interesting, however, that although there are fewer cotyledons in subsequent pregnancies they are significantly larger, suggesting some fetal influence upon the growth of the cotyledonous mass. Exposure of pregnant sheep to heat also leads to significant reduction in fetal size.

In mice, an explanation of immunological influences upon placental growth has been sought by crossing different strains and comparing the results with placental development of inbred strains but so far the results have been conflicting. Blastocyst transfer, the removal of some fetuses in litter-bearing species, the introduction of teratogens, and the induction of infectious diseases are other methods that have been employed to answer specific questions. Severe maternal nutritional deprivation is associated with pronounced reduction of fetal growth. Fetal hypophysectomy, thyroidectomy, or nephrectomy are associated with stunted growth in sheep. The mechanisms of these effects remain undetermined. Any attempt to relate the results of these ablative studies to the development of the human fetus is fraught with difficulty. The human models of anencephaly or ateliotic dwarfism cannot currently be used in a comparative context because of lack of information on the intrauterine hormonal environment.

The value of many of the animal experiments performed so far is limited in that they delineate deleterious influences rather than clarify the physiological mechanisms that normally control fetal growth. Moreover, in most cases it has not been possible to draw rigorous conclusions as the insults have not been sufficiently quantitated. The extrapolation of the results to human pregnancy is further limited by marked differences in the endocrine parameters of pregnancy, and the uterine and placental structure and blood supply in the species employed. Animal studies have also not provided a model to permit investigation of severe pre-eclampsia, one of the commonest causes of human fetal growth retardation.

¹ Wigglesworth, J. S. (1966) Fetal growth retardation, *Brit. med. Bull.* **22**, 13.

Further studies are needed in many areas. Methods must be developed in animals to assess placental perfusion characteristics (fetal and maternal) more adequately, with the ultimate goal of developing a satisfactory human placental function test.

At present our knowledge is also grossly inadequate with respect to the existence of fetal regulatory mechanisms that directly affect placental growth and transfer. Further animal studies in this area are needed.

Pathological findings in placentas of subhuman primates suggest that some species may have lesions similar to those of pre-eclampsia. Further efforts should be made to elucidate these findings by studying primate pregnancies.

At present the mechanisms that control trophoblast expansion or its endocrine performance are not understood, although some experiments suggest participation of immune mechanisms in the former. Since these relate closely to placental development, studies must be undertaken to further our knowledge in this area.

4. ROLE OF FETAL MATURATION IN THE INITIATION OF LABOUR

Despite considerable research,¹ the mechanism for the initiation of parturition in man is still not understood. However, recent investigations, stimulated by genetic and teratological observations in cattle and sheep, have demonstrated the active involvement of the fetus in determining the duration of pregnancy. They showed that ablation of the pituitary of the fetal lamb led to an indefinite prolongation of pregnancy, whereas infusion of adrenocorticotrophin (ACTH) or glucocorticoids into the fetus resulted in premature parturition. This work focused attention on the possibility that the fetal pituitary-adrenal system was involved in the initiation of parturition. This hypothesis was supported by an observed rise in plasma corticosteroid concentration in the fetus during the 7-10 days before birth. This rise is unrelated to maternal plasma corticosteroid concentration but was subsequently shown to result from an increased secretion of cortisol by the fetal adrenal gland together with an increase in cortisol-binding globulin in fetal plasma. In the rhesus monkey, ablation of the fetal pituitary gland led to a significant prolongation of gestation with marked involution of the fetal zone of the adrenal and differentiation of the adult portion.

Evidence that the fetal pituitary-adrenal system plays a similar role in the initiation of labour in man is not yet conclusive. General opinion favours the view that anencephaly with adrenal hypoplasia predisposes to

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1971, No. 471.

prolonged pregnancy, provided it is not associated with hydramnios. Difficulties have arisen because of attempts to extrapolate from observations on human anencephaly in which the absence of anterior pituitary function was not demonstrated.

Information about the functional maturation of the human fetal adrenal gland has been gained by measuring glucocorticoid levels in cord serum at delivery. Fetal cortisol levels are low in early gestation but by late gestation the values were found to have tripled in the umbilical arterial blood of fetuses in women who had not had a spontaneous labour. These blood samples were taken from the fetus at elective Caesarean section and after birth from infants born following labour induced with oxytocin. Cortisol levels were, in general, higher in those infants born following spontaneous labour, regardless of the mode of delivery; the significance of this difference is not known. Cortisol crossing the placenta is largely converted to cortisone and higher levels of 17-hydroxycorticosteroids in cord serum are mainly related to maternal stress. In other words, cord serum cortisol largely reflects fetal adrenal function, whereas fetal cortisone levels are reflections of the amount of maternal cortisol crossing the placenta. Although no reports of fetal plasma ACTH levels are available in the human, it has been shown that there is a significant decrease in the concentration of ACTH in the human fetal pituitary gland between 11 and 23 weeks gestation. It would appear that the maturation of the pituitary-adrenal system in man commences earlier in gestation and develops more slowly than that of the sheep. Vasopressin may potentiate ACTH release induced by corticotrophin releasing factor. Extremely high concentrations of vasopressin and oxytocin have been observed along with high cortisol levels in the cord blood of infants born after spontaneous labour and delivery.

Whereas glucocorticoid infusion in fetal sheep and goat initiates labour and produces striking hormonal changes in the mother and fetus, most evidence suggests that in man glucocorticoids may be ineffective in inducing labour although this question deserves further study.

Activation of the fetal adrenal gland may initiate labour by the increased production of estrogen precursors. Estrogens play a key role in the initiation of parturition in sheep and goats. In the fetal lamb near term, corticosteroids stimulate the placental synthesis of estradiol-17 β , which in turn stimulates the production of prostaglandin F_{2a} in the uterus. In human pregnancy there is also a steady increase in estrogens that may stimulate synthesis of prostaglandins, although the temporal relationships differ.

The appreciation of the mechanisms involved in initiating premature labour is of great clinical importance. Animal observations have shown that insults to the fetus, such as hypoxia, stimulate release of fetal ACTH, vasopressin, catecholamines, and renin. If the stimulus is prolonged or repeated it may result in premature parturition; closer to term the response

of the fetal adrenal gland to ACTH is more rapid. Other animal experiments indicate that maternal conditions sufficient to elevate maternal glucocorticoid levels over a period of time may precipitate parturition.

In the light of these animal studies, there are many clinical conditions of mother and fetus that might be expected to activate the fetal pituitary-adrenal system and to be associated with premature labour. These would include disorders that result in restriction of placental blood flow, such as severe pre-eclampsia, or those that affect the fetus directly, such as infections.

Further animal studies are needed to investigate the maturation of the mechanisms controlling fetal ACTH secretion and the nature of the stimuli responsible for the initiation of parturition.

Since ACTH release may be pulsatile and have a diurnal variation, frequent blood sampling is required and, therefore, a more sensitive ACTH assay is needed. For such studies the fetal sheep and goat are appropriate, but attempts must be made to determine whether similar mechanisms are operative in nonhuman primates. A promising approach to this problem may be the use of specific teratogens in animals to produce conditions simulating human anencephaly.

Further animal studies of the mechanisms by which maternal insults affect the fetus and lead to premature parturition are needed. The goat may prove a good experimental model because of its greater natural tendency to abort.

Information is needed on the functional maturation of the pituitary-adrenal system in man. The measurement of hormone levels in aborted fetuses, or in blood samples collected from infants during premature or term labour and from anencephalics may give valuable insight, particularly if they can be correlated with histological observations.

Ultimately, premature labour is the result of myometrial contraction and one must, therefore, not lose sight of the importance of gaining an understanding of the cellular events in the myometrium. Studies are needed in man and animals to elucidate the interrelationships between steroid hormones, prostaglandins, catecholamines, oxytocin, vasopressin, and the adenylcyclase system of the myometrium.

A collaborative study should be undertaken in man and one or more nonhuman primates to determine the effects of synthetic ACTH and glucocorticoids in initiating labour.

5. RESPIRATORY SYSTEM

A mature respiratory system at birth is characterized by the rapid establishment of the ability to respond to the gaseous metabolic demands of the infant over a reasonable range of environmental temperatures with

an adequate safety margin. Effective function depends on the development of both central and peripheral mechanisms, as exemplified by adequate responsiveness to changes in P_aO_2 , P_aCO_2 and temperature variations, by the development of adequate pulmonary surfactant and a tireless respiratory musculature, and by adequate perfusion of the pulmonary vascular bed.

Immaturity of this system may lead, amongst other things, to the respiratory distress syndrome (RDS) and to excess perinatal morbidity and mortality from asphyxial episodes due to recurrent apnoea. The therapeutic value of glucocorticoid administration to the mother to accelerate maturation of the lung and so avoid RDS in her offspring is currently under investigation. It is also possible that immature respiratory responses contribute to the "sudden infant death" syndrome.

The maturation of the fetal lung can be assessed antenatally with respect to development of pulmonary surfactant by measuring certain constituents of amniotic fluid. Measurement of certain proteins, such as α_1 -antitrypsin in blood taken from the umbilical cord, or measurement of pressure-volume relationships of the lung immediately after birth may also be indicators of the risk of subsequent RDS. These assessments must be made against a background of clinical information, including a knowledge of familial incidence of respiratory distress in previous siblings, the occurrence of fetal asphyxia, the duration of ruptured membranes, and the nature of the drugs given to the mother, particularly steroids, sympathetico-mimetic amines, and morphine derivatives.

Immaturity of the respiratory system is a major contributing factor to mortality and morbidity of the preterm infant. Effective therapy will depend on a better understanding of the structural and the functional development of the lung. Studies on surfactant production and its control have been made on a number of species including sheep, rabbits, goats, and monkeys. The apoprotein of pulmonary surfactant has been identified and comparative studies are in progress of its effect on maturation of the lung. The first evidence of accelerated maturation of the lung following administration of glucocorticoids was obtained in rabbits, a species in which surfactant develops during a short period in the last 3-4 days of gestation.

Investigation of the basic physiology, pathology, and pharmacology of fetal breathing has been and is being undertaken in sheep. This species is very suitable in that direct quantitative methods can be run continuously night and day. The fetus is of a size sufficient to provide blood samples for gas and hormone analysis and permit simultaneous recording of the electroencephalogram and of the swallowing responses. There is a clear difference between the different stages of maturation. Observations in sheep have clearly established maturational development of the fetal breathing system in terms of frequency and depth of breathing movements. There are also indications in both sheep and man that the movements become more

regular with increasing fetal age. Fetal respiratory movements have been recorded in man from the twelfth week of gestation using ultrasound techniques. This method may give valuable general information about fetal health as well as specific information on the development of respiratory movements.

Animal studies will continue to be the main source of fundamental information on the development of the respiratory system but considerable interspecies variation has been observed. The pattern and duration of fetal respiratory movements in sheep and man are known to be different, although this may prove to be relatively unimportant. Similarly, the enzyme pathways for surfactant production may vary in different species.

Further investigations of many topics are needed. These include evaluation of the factors that control the production of surfactant and its passage from the lung through the pharynx into the amniotic fluid; the timing of the developmental and structural stages; the development of autonomic control; inducer-receptor sites and sensitivity; and the mechanisms by which glucocorticoids accelerate maturation. It is also necessary to investigate the hazards of administering glucocorticoids to the fetus.

The ultrasound method of recording fetal breathing deserves further evaluation in man. Well designed trials in several centres are needed to assess its usefulness in comparison with measurements of heart rate and observation of other fetal movements. Preliminary work suggests that in man, as in sheep, normal breathing movements are arrested by fetal hypoxia and hypoglycaemia; the appearance of gasping in the fetus may have a sinister prognostic significance antenatally or during labour. While further work on man is needed, there are several features of the respiratory system that cannot be studied in man, for example, the effect of prolonged interruption of fetal respiratory movements on postnatal survival; the identification of drugs potentially dangerous to the respiratory system; the assessment by means of nerve section or hypophysectomy of the influence of other systems, neural or endocrine, on respiratory development.

Experiments on pregnant rats subjected to unilateral pneumectomy indicate that disturbances in the respiratory system of the mother may have detrimental effects on the development of that system in her progeny (similar data were obtained with other organs including kidney, liver, spleen, thyroid, adrenals, and with the immune system).^{1, 2} The implications of these observations are of considerable importance. Further animal studies are required to elucidate the mechanisms involved and to explore the possible application of these observations for the benefit of the developing human fetus.

¹ Murashova, A. A. (1966) *Arh. Anat., Gistol. Embriol.*, **8**, 43-47.

² Vyazov, O. E. & Khodžaev, S. H., ed. (1973) *Rukovodstvo po immunologii*, Moscow, Medicina.

An understanding of respiratory frequency and rhythms and the role of reflexes arising from the airways is complex at any age; the unusual patterns found in infants deserve study with modern noninvasive techniques in order to understand apnoeic episodes in infants. Such studies may also give rise to information that may be applicable to mature individuals.

6. CARDIOVASCULAR SYSTEM

Before birth, efficient functioning of the cardiovascular system assists the fetus to withstand a moderate degree of hypoxia and hypercapnia that may result from a variety of antenatal pathological conditions and may occur during abnormal labour.¹ Under such conditions cardiac output is maintained or increased and there is a redistribution of blood flow, dependent partly on aortic chemoreceptor stimulation and partly on local reactions to changes in blood gas tensions; blood is diverted from the less vulnerable tissues (limbs, kidneys, gastrointestinal tract, and lungs) to those that are essential (heart, brain) with maintenance or increase of flow, dependent on arterial pressure, to the organ of gas exchange (the placenta). More severe asphyxia is associated with a further rise in arterial pressure, an increased diversion of blood to essential organs, and slowing of the heart, which has proved a useful diagnostic sign in man. There is also a large rise in fetal plasma catecholamines, vasopressin, and corticotrophin (ACTH). All but the local reactions to asphyxia are dependent on maturation of the central control mechanisms. The metabolic reserves on which the heart and brain depend during oxygen deprivation and the capacity to mobilize glycogen from the liver also vary with maturity.

In man at birth, a mature cardiovascular system is characterized by a high, well maintained cardiac output, a fluctuating heart rate that is less than that early in gestation, and an arterial pressure appropriate to age. Its capacity for adaptation, though limited, is sufficient for normal purposes; for example, it can support the increased blood supply to the organs of extra thermogenesis on exposure to cold (to a rate of gaseous exchange of about 15 ml of O₂/kg per min) and blood loss within limits. It is not yet certain whether in man the normal competence of the circulation after birth depends on relative maturity of the autonomic nervous system or, as in the rabbit, of that of the renin-angiotensin system. It certainly depends on normal closure of the fetal circulatory channels at or soon after birth, especially the umbilical vessels and the ductus arteriosus. This competence is also dependent on the proper expansion of the lungs and well maintained respiratory ventilation and, to some extent, on the volume of blood transferred from the placenta to the fetus at birth.

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1965, No. 300.

Failure in any one of these respects can lead to clinical evidence of cardiac failure—for example, in persistent patency of the ductus arteriosus, in hypovolaemia or excessively high haematocrit, and in pulmonary hypertension. In addition, pulmonary haemorrhage, in some instances described as fulminating pulmonary oedema, can occur, especially in immature small-for-dates infants. Intraventricular haemorrhage is seen in many preterm babies for reasons that are uncertain, but has been attributed by some to elevated cerebral venous pressures.

Progress towards the identification after birth of the pathogenic mechanisms of these clinical conditions has been slow. This is due to the lack of precise methods of measuring repeatedly the cardiac output and its distribution. Inadequate methods for evaluating the maturation of sympathetic innervation and of cardiovascular reflex mechanisms in the fetus and newborn have also impeded progress. Indeed, before birth it has been possible so far to record only the heart rate. The beat-to-beat variations and changes with uterine contractions have proved useful in fetal monitoring before and during labour, but measurement of heart rate cannot be regarded as other than a first step towards the rigorous scientific analysis of prenatal cardiovascular pathophysiology.

Several tests of the safety margin provided by the cardiovascular system of a human fetus *in utero* have been explored, the margin being judged by the changes in fetal heart rate on maternal exercise, exposure to 15% oxygen, or on induction of uterine contractions by oxytocin infusion. The results of these tests and of a method based on the ability of the fetus to excrete *p*-aminohippuric acid into the amniotic sac after maternal administration are still being assessed. A good, direct method of identifying specific high-risk pregnancies from those selected on general epidemiological grounds would be useful.

Only the sheep has been used prenatally for extensive cardiovascular studies of blood pressure, heart rate, the distribution of cardiac output, reflex responses to a variety of controlled perturbations (for example, cardiac slowing in response to a sudden elevation of aortic pressure or responses to maternal hypoxia or partial cord occlusion *in utero*), and for studies of changes in circulating blood catecholamines, vasopressin, and components of the renin-angiotensin system. Basic data are available from rhesus monkeys, dogs, and cats. "Chronic" preparations have been made on the fetus *in utero* in sheep and goats and should be practicable in the baboon with telemetry.

So far as the maturation of the cardiovascular system is concerned, the sheep proved an accurate model to show the principal features of the changes in the circulation at birth, subsequently observed in man, both anatomical and functional. Based on the expectation that a similar sequence of functional maturation will be present in these two species, irrespective of the incident of birth, animal work has continued to concentrate on the

sheep : the similarity is not certain, however. It was long ago pointed out that there was no need for the output of the two ventricles to be identical in fetal life and recent evidence in sheep suggests that they are not normally identical, but change with physical states. After birth, when the fetal circulatory channels close, the outputs of the two ventricles must approximate. Postnatal failure in this respect may contribute to cardiac failure. It will be difficult to determine if this is so in man.

In view of its relatively large brain size, the monkey might be a better model for studying the distribution of the circulation, particularly to the brain. In monkey, as in man, the brain is over 10% of the body weight at birth, in the sheep it is only a little over 1%. Consequently, a much larger fraction of the cardiac output may go to the primate brain.

Nothing appears to be known of the factors that limit, regulate, or accelerate maturation of the circulation, the perfusion of tissues, and the responses to hypoxia. It is only recently that it has become possible to conduct longitudinal studies in fetal sheep. Such as they are, they indicate a need for further investigation, for example, to seek explanations of the abrupt changes in arterial pressure observed during 3-week periods of continuous study. The response of the ductus venosus to *in vivo* hypoxia deserves investigation.

The factors affecting closure of the ductus arteriosus, particularly in the preterm infant require further investigation. Some of these observations might be made *in vitro* on material obtained from premature animals or human infants at postmortem. Further information may be gained from observations on experimental animals born prematurely following administration of synthetic ACTH to the mother (sheep and cattle) and possibly from animals with a genetically determined patent ductus arteriosus (dogs).

There is a strong case for seeking animal models for pulmonary and intraventricular haemorrhage in the newborn, in order to define the pathogenic causes. Investigation of both problems would include evaluation of the contributions of coagulopathies and asphyxia as well as perfusion. Pulmonary haemorrhage occurs in newborn rabbits that fail to respond to cold and further study of this condition may help evaluate the problem in man. Spontaneous intraventricular haemorrhage has not been observed in experimental animals.

7. THERMOREGULATION

Within a few hours of birth the full-term human infant maintains thermal stability over the range of ambient temperatures to which it is usually exposed. In response to cold exposure, heat loss is reduced by vasoconstriction and postural adjustments and heat production is increased to as much as 3 times the resting level, mainly by thermogenesis in a special

heat organ, i.e., brown adipose tissue. In response to warmth, heat loss is increased by peripheral vasodilatation and sweating. However, the average full-term infant, unlike the adult, is unable to maintain a thermal equilibrium even temporarily at environmental temperatures as high as body temperature. The ambient temperature range over which the infant can control body temperatures varies with body weight, age, state of nutrition, and health. It is greatly increased by clothing.

The newborn differs from the adult in that the core temperature is not held within a very narrow range. During cold exposure the core temperature is allowed to fall even when the infant has a reserve capacity for heat production. Similarly, the core temperature rises before sweating begins.

The infant born at 30 weeks gestation responds to cold by vasoconstriction and increased thermogenesis, but thermal control in cool environments is limited by the large surface area relative to body weight and poor thermal insulation. The response to warm ambient temperatures is also poor owing to delayed development of sweat glands.

In a number of nurseries caring for preterm infants, a fall in mortality has been reported when care has been taken to provide extra warmth. Steps to prevent heat loss must be initiated immediately after birth, whether in the delivery room or the operating theatre. The increased mortality observed without this care has not been associated with any recognizable pathological changes. To maintain thermal stability in a cold environment the body must produce extra heat and therefore the body's furnaces compete with the remaining body functions for the supply of oxygen and fuel. This competition increases the problems in preterm infants with difficulty in establishing an adequate pulmonary gaseous exchange and in infants with limited fuel resources. Too warm an environment may also be hazardous, for sharp rises in ambient temperature can induce apnoeic episodes.

Some indication of the thermoregulatory capabilities of the preterm infant can be gained by recording ambient and body temperatures. However, other methods are available that measure rates of heat production and these make it possible to estimate the rates of heat loss by conduction, convection, radiation, and evaporation.

The thermoregulatory responses of many newborn mammals have been studied. For example, smaller species, such as mice and rats, have been used to observe the effects of rearing many generations in cold environments. Under such conditions the newborn litters stay in the nest and need frequent feeding. The relatively immobile and hairless newborn rabbit is also nest dwelling and one of a litter but feeds only once or twice each day. Rabbits have proved useful in the study of the biology of brown adipose tissue. The guineapig, whose motor behaviour is advanced at birth, has been studied to evaluate the relative roles of shivering and non-shivering thermogenesis. The newborn guineapig, although of good size,

has relatively poor thermal insulation and is unusual in that the fat stores in white or brown adipose tissue are small. In a cold environment it therefore depends, before feeding commences, on shivering thermogenesis supported by its carbohydrate reserves: its climatic physiology has been extensively investigated. The lamb soon after birth has thermoregulatory responses that compare well with those of the adult sheep. This is not true of lambs born before term following synthetic corticotrophin administration to the mother. The climatic physiology of full-term lambs has also been thoroughly evaluated.

Animal studies have contributed considerably to our understanding of the thermoregulatory systems in the human infant. However, the evaluation of the effectiveness of these systems can only be made in the nursery, for this depends, among other things, on the physical characteristics of the infant and the environment.

Further investigations in animals of the factors that enlarge or restrict the infant's capacity to respond to heat or cold would be valuable. For example, little is known of the development of thermoregulatory mechanisms *in utero*; of the maturation of sensory systems; or of factors determining the growth and fat content of brown adipose tissue. Neither the mechanisms that initiate thermogenesis at a cellular level after birth nor the factors that lead to the disappearance of nonshivering thermogenesis and the rise of shivering thermogenesis are understood. Experimental data from neonatal animals show that ambient temperatures can have profound effects on the growth and development of many organs, but the evidence is patchy and most studies have concentrated on the short-term effects.

The optimal thermal environment for sick infants may well be that which reduces extra heat production to a minimum and therefore reduces oxygen and fuel losses, so that adequate amounts are available for both maintenance and growth. However, the effects of holding a human infant in a constant thermal environment have not been studied and the appropriate way to introduce cold stress has also received little attention.

It would be of great value to know more about the mechanisms by which a rise in ambient temperature induces apnoea and to be able to recognize some signs that could act as danger signals when such an event is imminent. Since apnoeic spells have been observed to follow warming of the exteriorized fetal sheep, the lamb may prove to be a suitable animal model.

There can be no doubt that cold exposure is a powerful stimulus to respiration, cardiac output, and peripheral circulation in the newborn. It could be argued on these grounds that short periods of cold exposure may be beneficial to those infants who can tolerate it, but clearly further investigations are required.

In every situation where preterm and sick infants receive care, it is important to evaluate the effective thermal environment and the infant's

response to it. Information and techniques are available to make these assessments and considerable benefit would follow their wider application.

8. CENTRAL NERVOUS SYSTEM

Primates, including man, differ from other altricial¹ mammals in that their sensory functions are relatively mature at the time of birth. In contrast, the motor system is remarkably immature. Maturation of the central nervous system continues long after birth and is not complete until puberty. In particular, neocortical functions are slow to develop. Yet the anatomical framework of subsequent neocortical development is established prenatally. In both sensory and motor systems individual neuronal function is present at an early stage of prenatal development. With regard to sensory function there is a fixed order of sequential development of different modalities that is common to most species, as evidenced by both electrophysiological and behavioural studies.

There are data available on the ontogenesis of the sensory systems of different species, as well as on cortical function as displayed, for instance, in the electroencephalogram. Some information is also available on the development of spinal-reflex patterns. Very little is known about the development of motor function, particularly with regard to supraspinal motor control. The evolution of sensory motor integration in any species is poorly understood.

Immaturity of the central nervous system at birth does not in itself create great clinical problems. The incident of birth does not place special adaptative strains on the central nervous system as compared with, for example, the cardiovascular and thermoregulatory systems. The presence even of severe brain malformation or damage in the neonate (e.g., hydranencephaly) is compatible with survival. However, dysfunction of the central nervous system in the neonatal period constitutes a major clinical problem and a search must be made during the prenatal stages of development for etiological factors in central nervous system dysfunction. In this context epileptic disorders, motility disturbances, impairment of sensory function, and the syndrome of minimal brain dysfunction are of significance. Near term the major features of neuronal organization have usually been established in the human infant. Studies devoted to the exploration of the normal course of neuronal development and its pathophysiology *in utero* cannot be performed on man, and must therefore be explored on experimental animals.

Although extensive observations have been made on postnatal behaviour and development in man and animals, standardized and quantitative

¹ In need of prolonged nursing.

diagnostic methods of detecting signs of central nervous system dysfunction at an early postnatal stage are still required.

Interpretation of dysfunction of an immature central nervous system should take into account the fact that the functional maturation of a given system, afferent or efferent, does not occur uniformly and that different parts may develop independently of each other.

Observations on the normal course of neurological development are necessary in order to identify periods of accelerated development or "growth spurts". It is possible that these phases of neurogenesis may be especially susceptible to changes in the internal or external environment. These studies of the normal development of the central nervous system should take into account neurochemical aspects, with particular regard to the central neuro-transmitter systems.

Interference with the normal sequence of neuronal development in experimental animals by surgical and chemical lesions within the central nervous system, exposure to measured degrees of hypoxia, and endocrine changes may give clues to the genesis of, and mechanisms for the development of, brain dysfunction. The role of undernutrition and malnutrition in this process should be emphasized. With regard to the motor system, such an experimental approach is particularly necessary for the understanding and treatment of motility disorders. Experimental manipulation of the developing fetal nervous system might well be directed towards a study of well-defined sensory or motor functions as well as to evaluation of complex behavioural and cognitive parameters.

It may be of practical importance to investigate further the possibility of accelerating functional development during early prenatal life by experimentally manipulating the environment. Experiments during the postnatal period in animals have shown that an increase or decrease in sensory stimuli may alter the rate of normal development but few relevant data are available concerning the prenatal period. Quantitative studies of the effects of malnutrition on pre- and postnatal maturation of sensory functions are pertinent. Such experiments should be designed on a long-term basis in order to assess the reversibility of retarded maturation.

The etiology of the different types of epilepsy may be related to disturbances in the prenatal period. The nature and incidence of such factors are insufficiently known. Long-term animal studies may help to identify epileptogenic mechanisms established early in development.

The syndrome of minimal brain dysfunction is an important cause of maladaptation in children and may be a result of exposure to different degrees of hypoxia during the fetal and neonatal periods. Subhuman primate models may be used to study such a marginal neurological deficit, primarily affecting cognitive functions, to extend the observations made on man. There is a great need for investigations to establish a correlation

between minimal brain dysfunction and morphological changes, in particular, changes affecting the neocortical neuronal elements.

Most studies on the functional development of the central nervous system have been confined to the postnatal period and have usually been performed on cats, rats, guineapigs, and rabbits. Data on the early prenatal phases of development have been gathered from the sheep, the guineapig, and to some extent from the dog. Sheep and guineapigs are suitable as experimental animals for studying the early stages of neurogenesis and techniques for both acute and chronic fetal preparations have been developed. There are great interspecies differences, in particular with regard to the organization of supraspinal motor control as well as to integrative neocortical functions. Therefore, data derived from experiments on non-primates can often not be directly applied to man and this should be taken into account when selecting the animal species to be used as a model.

9. DEVELOPMENT OF THE IMMUNE SYSTEM

The immune system of a normal newborn is qualitatively complete, and is lacking only in antigenic experience. It is composed of a very large number of highly specialized and mobile cells that act in a coordinated way to recognize, inactivate, and eliminate potentially harmful invaders. These interactions may be summarized as follows.

Both fixed and wandering cells of the reticuloendothelial system may aid in bringing foreign substances into contact with members of specific clones of thymus-derived T lymphocytes that have complementary antigen receptors on their surface. This interaction results in activation of the T cell and sets into motion a number of metabolic events. Among these is the production and release of biologically active substances collectively called lymphokines. The lymphokines defined so far include capillary permeability factor, macrophage chemotactic factor, macrophage activation factor, interferon, cytotoxic factor, factors that recruit other lymphocytes into the fray, and T cell factors that can curb the immune response.¹ In addition, the T lymphocytes are stimulated by antigen to enlarge and divide, thus expanding the size of the clone of cells responding to the particular antigen. Cellular immunity, mediated by T cells and macrophages, is especially needed for the control of many virus and fungus infections, and is important in relation to the prevention and control of neoplasms.

The induction of humoral immunity involves antigen-triggering of a finite number of clones of B lymphocytes, the other major class of lymphoid cells that is derived from the bursa in birds, and a still undefined

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1973, No. 519.

equivalent in man. The activation is via interaction of antigens with antibodies bound on the membrane of the B lymphocyte. Activated T lymphocytes also cooperate in triggering B lymphocytes to develop into mature antibody-producing and -secreting plasma cells. The immunoglobulins secreted combine with circulating antigens and in turn activate the complement system, which is an interacting group of 11 circulating proenzymes. Among the biological activities of the enzyme fragments generated by the cascading interaction of complement components are dilatation and altered permeability of blood vessels, chemotaxis of leucocytes, facilitation of phagocytosis of the antibody-coated invader, and lysis of membranes of antibody-coated cells. Humoral immunity is of major importance in control of bacterial infections and detoxification of toxins such as endotoxin. Termination of the immune response requires the effective elimination of antigen by circulating antibodies. The initial encounter prepares the individual for a faster and greater cellular and antibody response upon a second encounter with that antigen. An alternative outcome of the introduction of antigens may be the induction of a state of specific immunological tolerance; integrity of this mechanism is required for the prevention of autoimmune injuries.

By mid-gestation, the human fetus displays functional heterogeneity of T and B cells, phagocytic cells, and synthesis of complement components. Antigenic experience after birth leads to rapid expansion of selected clones of T and B cells and production of circulating antibodies. During this period of life, the umbrella of maternal (IgG) antibodies affords some protection.

Invasion of the very young fetus by pathogens such as rubella virus, cytomegalovirus, and *Toxoplasma gondii* often results in a chronic infection, in which defective differentiation of the immune system itself may be manifested by impaired cellular and humoral immunity. Results of studies in mice suggest that circulating antigen-antibody complexes may initiate some of the widespread organ damage in infants with intrauterine infections. Information obtained in many animal species indicates that persisting viruses may in certain instances lead to the development of leukaemia or other malignancies at a later date. Acute infections in the newborn, especially the premature infant, may be a more frequent consequence of immunological immaturity; the common occurrence of diarrhoea and sepsis due to intestinal microorganisms suggests that immunological monitoring of this portal of entry is not well developed at birth.

Practical techniques are available for direct identification of T and B cells, although some of these are not yet well standardized. It is also possible to measure their responses to antigens or mitogens and some of their specific end products, for example, immunoglobulins.¹ Complement

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1973, No. 519.

components and phagocytosis can also be measured accurately. There is, however, no good way of sampling these blood elements before birth.

An immune system capable of specific adaptive responses can be found in all vertebrates. Animal studies at all phylogenetic levels of existing vertebrates have been and should continue to be useful in understanding basic biological principles of the immune system. Specific examples of some animal models needed are listed below.

It is worth emphasizing that demonstration of similarity of immunological mechanisms in several animal species may increase the likelihood that the same mechanism is of basic biological importance and therefore might be similar in the human. The immuno-deficiency diseases of man have been especially helpful in the integration of information obtained in animal studies.

A mammal in which the B cell system can be selectively eliminated (as in a gammaglobulinaemic human and chick) is needed for studies on the nature of the antigen receptors for T cells. Immunoglobulin receptors on T cells make it difficult to resolve this question using cells from normal animals. The information is needed to understand tolerance, T lymphocyte heterogeneity and its developmental basis, cellular immunity, and regulatory interactions between T and B cells.

The site at which B lymphocytes are spawned in mammals is still uncertain; it must be identified before the generation of the clonal diversity of B lymphocytes can be understood.

The effect of depriving premature primates of their normal supply of maternal antibodies needs definitive study.

The neonatal development of the secretory antibody (IgA) system of mucous surfaces needs further elucidation. Early oral immunization as a means of enhancing development of this system could have important practical benefits in selected environments. Conversely, the protective value of antibodies in human colostrum and milk also deserves further study.

There is a need for further study of the effects of nutritional deprivation and chronic hypoxia on development of the immune system.

The effect of hormones, particularly adrenal glucocorticoids, on the developing immune system and the mechanisms whereby this effect is exerted appear to be important issues.

Extended study of the cellular and humoral response in neonates as evidence of intrauterine infection is recommended.

The influence of the maternal immune system on the maturation of fetal immune systems and on development of other fetal organs deserves further exploration and may prove to be of considerable importance.

10. DEVELOPMENTAL PHARMACOLOGY

Drug disposition is determined by absorption, distribution, biotransformation, elimination, genetic composition of the host, and receptor affinity. The fetus differs from the premature and mature infant in this regard since the first can also utilize the resources of the maternal organism via the placenta. Following parturition, however, the neonate must rely solely upon its own incompletely developed mechanisms for the disposition of pharmacologically active molecules. Thus, absorption of particular drugs from the gastrointestinal tract may be impaired, distribution may occur into membrane-bound compartments that are less permeable during later life (e.g., central nervous system), biotransformation is reduced, and the clearance of drugs, nonmetabolized or metabolized, via the kidneys is significantly less than in children or adults.

Clinical drug therapy is, in most circumstances, determined by the effect(s) a specific chemical compound will exert upon the host. Since maturation of the host may alter its reactivity to drugs, any therapeutic intervention requiring administration of pharmacological agents to the fetus or neonate must be evaluated critically. The adverse effects of drugs administered during human gestation may be manifested as alterations in cellular development or as modifications in cellular function that may persist postnatally. Immaturity of drug disposition processes in the human fetus and neonate is generally, but not always, associated with an increased sensitivity to pharmacological agents. Some clinical examples of such phenomena seen in the neonate are: hyperbilirubinaemia (sulfonamides); sedation (reserpine, methyl dopa); and neurotoxicity (gentamycin, ethacrinic acid). Antibiotics that cross the placenta may significantly influence fetal development, since they have the potential to inhibit cellular growth. Other therapeutic agents have been shown to induce or restrain maturing systems and this potential hazard must be appreciated.

The techniques that are available for quantitatively assessing drug disposition in fetal and neonatal tissue are generally complex. The most useful methods appear to be pharmacokinetic analysis, measurement of specific indicators of drug metabolizing capability (e.g., (+)-glucaric acid in urine), and identification of the metabolic products of drugs or endogenous substrates that have undergone biotransformation reactions (e.g., glucuronidation, sulfuration, *N*-dealkylation). The use of inert chemicals, with no apparent pharmacological action, or stable isotopes to assess both the number and the extent of utilization of discrete metabolic pathways requires further development.

It is important to determine which experimental animal constitutes the best model for investigating the effects of drugs upon the fetus and neonate. It seems unlikely that one species will be useful for all types of study. Large species differences in placental, fetal, and neonatal physiology exist, so

that no assumptions can be made without direct testing. The relevance and comparability of *in vitro* perfusion and *in vivo* studies are unresolved.

Only scanty information is available on the metabolism of drugs by human fetal liver and most statements regarding the presence of qualitative enzymatic deficiencies during ontogenesis are based on animal studies. It has been shown that many oxidative activities are barely detectable in tissues obtained from fetal or newborn animals (e.g., side-chain oxidation of hexobarbital, aromatic hydroxylation of acetanilide or naphthylamine, deamination of amphetamine, dealkylation of aminopyrine). Similarly, the human fetal liver is unable to oxidize *N*-monomethyl-*p*-nitroaniline, to hydroxylate benzo[*a*]pyrene, or to demethylate aminopyrine to any significant degree. In contrast, the degree of metabolization of aniline and ethylmorphine by human fetal liver is 40% and 35%, respectively, of that observed in human adult liver extracts. These experimental observations illustrate the importance of defining intraspecies variations with regard to the drug metabolizing activity of developing hepatic tissue. Observations in rodents would have been misleading as a guide to what happens in the human fetus and infant.

The fetal distribution of drugs may differ between species. Thus, the tissue localization pattern of phenytoin in the mouse, rat, and human is similar, whereas that of digoxin differs markedly in the rat in comparison with that in the sheep or man, which are virtually the same.

From the data available it is not possible to predict the most suitable single animal species for perinatal pharmacological studies.

Knowledge of perinatal pharmacology requires considerable expansion if incubation of the fetus and neonate in "a sea of drugs" created by iatrogenic or environmental factors is to be avoided. Among those areas that appear to deserve high priority for investigation are the following: studies on the mechanisms regulating drug disposition in the materno-placento-fetal unit; the effects of drugs on normal fetal and neonatal function; the influence of genetic factors and of nutritional and disease states on fetal and neonatal drug disposition; the physicochemical characteristics of pharmacologically active molecules and their influence on placental transfer and disposition by the fetus and neonate; the development of improved ultramicro-methods for the quantitative and selective determination of drugs and their metabolites in the small samples available from the fetus; and improved epidemiological monitoring of the patterns and consequences of drug usage during pregnancy.

Despite the difficulty of deciding which animal species is most suitable for studies in perinatal pharmacology, it should be noted that the sheep, goat, and nonhuman primate are particularly useful for investigating the kinetics of placental transfer and the effects of drugs upon the developing cardiovascular, renal, endocrine, and nervous systems. Smaller animals (e.g., mice, rats, rabbits), which have a shorter gestation period and in

which highly inbred strains are readily established, have been employed extensively in the study of fetal drug distribution and metabolism as well as of thermoregulation and pharmacogenetics. Tissue cultures derived from animal and human species also appear to constitute valuable tools for assessing the influence of drugs upon fetal structures at the cellular and molecular levels.

11. EXPERIMENTAL ANIMALS : GENERAL CONSIDERATIONS

It is evident that much of the present knowledge of the biological events in the last third of pregnancy and the perinatal period has come from systematic studies on experimental animals, particularly sheep. The choice of animal for a given study clearly depends on the problem investigated, and some comments on this point appear under the discussions of the different systems. Patterns of development may be followed within any given species ; adaptation of size and function may be best studied by selecting species of different sizes ; and metabolic functions may sometimes be elucidated by choosing species with specific genetic characteristics.

The primary consideration that influences choice of an animal is its suitability for the particular investigation. Clearly placental function in man is best studied in man, or when not feasible, in an animal whose placenta closely resembles that of the human, such as a nonhuman primate. On the other hand, the physiology of the placenta of other mammals is of interest for its own sake and knowledge of different placentas may illuminate aspects of human function.

Other considerations of choice of animal for study, if several suitable species are available, include size and ease of study, the availability of related studies in the literature, length of gestation, ability to determine gestational age, health of the experimental animal, ease of breeding, accommodation, and handling, seasonal availability, and cost. In general, careful choice of species is the essential first step in biological investigations.

12. GENERAL RECOMMENDATIONS

(1) It would be valuable to investigate the maturation of fetal body systems in a wider range of species.

(2) Some information of use in the prevention of perinatal morbidity and mortality can only be obtained in man. The use of human fetal material has been valuable and should be continued with due regard to ethical considerations.

- (3) The cellular mediation of myometrial response and fetal organ maturation throughout pregnancy should be studied in animals and man.
- (4) The interaction of maternal and fetal organ systems in the pathogenesis of postnatal disability and defects in maturation should be explored.
- (5) The short- and long-term consequences of accelerated maturation require both experimental and clinical evaluation.
- (6) Quantitative studies of the nutritional needs for optimal organ development as well as the adverse effects of specific deficiencies should be studied in the fetus and newborn.
- (7) There is a need, in the near future, for a meeting of experts to consider the causes and prevention of spontaneous premature labour.

13. SPECIFIC RECOMMENDATIONS

Regulation of fetal growth and placental competence

- (1) The regulatory mechanisms governing growth and function of the trophoblast should be studied in detail.
- (2) Knowledge of placental function and its regulation by maternal and fetal factors must be further amplified by detailed physiological studies in experimental animals.

Fetal maturation in the initiation of labour

- (3) A collaborative study should be designed to investigate in man and one or more species of subhuman primate the benefits and risks to the fetus of using synthetic ACTH and glucocorticoids to initiate labour and to induce pulmonary maturation in the fetus.
- (4) Further animal studies should be undertaken to investigate the mechanisms by which maternal stress affects the fetus and leads to premature labour.

Cardiovascular and respiratory systems

- (5) Quantitative studies would be valuable to investigate the maturation of the systems regulating blood flow to the placenta and the fetal brain and lung, first in animals and later, if practicable, in man.
- (6) It would be desirable to develop animal models of neonatal pulmonary and intraventricular haemorrhage.

(7) Further studies are needed on the detection of pulmonary surfactant in amniotic fluid and on the regulation of its synthesis, secretion, and turnover in animals as a basis for application to man.

(8) Collaborative studies are required in man on the usefulness of measurements of fetal respiratory movements as an indication of health, both antenatally and during labour.

(9) The maturation of respiratory control mechanisms before and after birth should be studied in man and animals in relation to neonatal apnoeic spells.

Thermoregulation

(10) The effects of ambient temperature on organ growth and development should be studied.

(11) More basic information is required to identify the factors that determine the maturation of thermoregulation before and after birth.

(12) The information now available on the climatic physiology of the newborn human infant and on the techniques for assessing the effective thermal environment and the infant's response to it should be more widely disseminated and applied wherever care is being provided for preterm and sick infants.

Central nervous system

(13) More basic data on the normal development of central nervous system function should be sought in mammals, in particular with regard to the maturation of supraspinal motor control.

(14) It would be desirable to identify vulnerable periods in the development of the central nervous system and to assess the effects of experimentally induced changes in the external and internal environment. Special attention should be paid to factors that may be responsible for epilepsy, motility disorders, and the minimal brain dysfunction syndrome.

Development of the immune system

(15) It would be valuable to find a species of mammal in which the B cell system of humoral immunity can be selectively eliminated and the site of its production identified.

(16) The neonatal development of the secretory antibody system of mucous surfaces should be explored further to find means of protecting the newborn against infections that so often overwhelm it through these pathways.

Perinatal pharmacology

(17) The disposition and effects of drugs in the materno-placento-fetal unit and during early extrauterine existence should be investigated in man and animals. The influence of genetic factors and of nutritional and disease states upon these processes should also be assessed.

(18) The structure-action relationships of pharmacologically active substances in developing organisms merit study. Concurrent efforts to develop ultramicro-analytical procedures for the detection of these compounds might facilitate the development of therapeutic agents for use in the fetus and newborn.

(19) Prospective clinical studies should be established to determine the prescribing patterns, patient compliance, and consequences of drugs administered during gestation, labour, and in the postnatal period. Investigations in this area might include collaborative studies of the effects of anticonvulsants on nervous and endocrine function and of the effects of psychoactive agents on nervous function.

Annex

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