DISTURBANCES OF MITOTIC PROCESSES AND TERATISM

DOUW G. STEYN, B.Sc., DR. MED. VET., D.V.Sc.
Professor of Pharmacology, Medical Faculty, University of Pretoria, Pretoria

In recent years clinical and experimental evidence in connection with congenital malformations and foetal deaths has thrown much light on the causes of teratism. The information collected suggests to us clear indications as to the causes and prevention of a large percentage of malformations and foetal deaths.

In the course of the last 5 or 6 decades experimental embryologists have produced various types of malformations and monsters in plants and aquatic animals by disturbing mitosis in embryos by means of chemical substances. These experiments, and especially Gregg’s observations in connection with congenital abnormalities in the offspring of pregnant mothers who suffered from rubella, turned our thoughts to the possibility of teratism in man being caused by the action of certain drugs and poisons shortly before or during pregnancy. It has become increasingly clear that heredity is not the only factor which plays a rôle in malformations. Talbot and Grebe and Windorfer believe that most congenital abnormalities are not of an hereditary nature but are acquired in utero. According to Richet and Rymer congenital abnormalities occur in 1–2% of normal individuals and some 300 different types are known. These include not only abnormalities of the organs but also congenital troubles associated with (1) the endocrine system (myxoedema, congenital diabetes, thymic hypertrophy, a tendency to obesity) and (2) diencephalo-hypophyseal troubles (dwarfishness, infantilism, etc.). It is obvious that the type and degree of damage done to the embryo depend on the force of the insult, the stage of gestation at which it takes place, and the period during which it acts. However, recent studies on cortisone by Fraser and his associates have shown that this hormone is capable of destroying foetal tissues which are already fully formed (cleft palate).

It is proposed to deal here with congenital deformities and foetal death in so far as they are caused by (a) vitamin deficiencies and (b) toxic agents, including diseases and conditions which induce anoxia.

(A) VITAMIN DEFICIENCIES

Vitamin A. Hale observed that sows on a diet deficient in vitamin A gave birth to young showing microphthalmia or anophthalmia, and other malformations, including hare-lip, cleft palate, and accessory ears. These observations were made on sows fed on a vitamin A deficient diet for 150 to 200 days before mating and during the first 30 days of gestation. Supplementation of the diet with vitamin A and D after 30 days gestation failed to correct the above malformations. However, when these vitamin supplements were given to these sows throughout the whole period of gestation the offspring were normal. Zilva et al. made similar observations on sows on vitamin A deficient diets many years before. Investigations conducted by Warkany and Nelson confirmed these observations of Zilva et al. and of Hale. According to Wilson et al. vitamin A deficiency in the pregnant rat causes death of the foetus and various malformations in the eyes, the heart-blood-vessel system and the genito-urinary system. They were able to prevent foetal death and the abnormalities by the addition of 150 μg carotin/week to the diet before and during pregnancy.

Not only hypovitaminosis A but also hypervitaminosis A may produce foetal abnormalities. Rodahl (quoted by Cohlan) and Moore and Wang reported that excess of vitamin A in the diet of pregnant rats caused a decrease in the number of litters and foetal resorption in the uterus. These observations were further investigated by Cohlan. He administered per os to pregnant female rats 35,000 IU vitamin A daily from the 2nd, 3rd or 4th to the 16th day after coitus and found that the administration of excessive amounts of vitamin A to pregnant rats produces a diminished litter rate and characteristic malformations among the surviving young. The anomalies included extrusion of the brain, macroglossia, hare-lip, cleft palate and eye defects. The cranial deformity was consistent.

Vitamin B (Riboflavin). Warkany et al. and Giroud et al. describe malformations in the offspring of mother rats on diets deficient in riboflavin during pregnancy. The former workers found that the malformations in rats were not determined before the 13th day of gestation and that, up to that time, liver supplements to the maternal diet prevented their occurrence. However, once the malformations were established supplements to the maternal diet might prevent abortions but would not correct the malformations.
Vitamin B₁₂. Hogan and Richardson (quoted by Giroud and le Febvres-Boisselet) produced experimental hydrocephalus in rats which was in a measure attributable to vitamin B₁₂ and folic-acid deficiency. Lepskovsky and his co-workers studied the effects of vitamin-B₁₂ deficiency on viability, growth and reproduction in 3 successive litters of rats. There was high mortality in the young 2 to 3 weeks after weaning, even when they were nursed by normal mothers. Severe intra-uterine injury was shown by third-litter rats. The abnormalities became progressively severe in successive litters. Oleese et al. describe congenital anomalies in chicks due to vitamin-B₁₂ deficiency. In eggs laid by hens on a B₁₂-deficient diet there was a high mortality in the embryos at the 16th to 18th day of incubation. The most characteristic deformity was 'spindly legs' (myo-atrophy of the legs), which also had a haemorrhagic appearance. Of unusual frequency was the abnormal position of 'head between the thighs'. O'Dell et al. produced offspring with numerous physical abnormalities by placing female rats on B₁₂-deficient diets. Hydrocephalus occurred in approximately 23% of the foetuses. There was also a high mortality in the young rats. Weekly injections of 1.0 μg. B₁₂ into female rats, before and during gestation, prevented hydrocephalus in the young. It appeared that damage to the foetus of mothers on B₁₂-deficient diets occurs at the 12th to 14th day of gestation.

Folic Acid. According to Nelson and Evans (quoted by Giroud and le Febvres-Boisselet) folic-acid deficiency in the pregnant rat causes absorption of some embryos and diminution in the sizes of the survivors. As folic acid is synthesized in the rat's intestine, deficiency of this vitamin in the rat can be produced only by preventing such synthesis. This was done by succinylsulphathiazole in the pregnant rat. The resulting deformities in the foetuses included coloboma, harelip, big facial clefts, nasal atrophy, coelosomia and ectocardia.

Riboflavin. Piccioni et al. kept mother rats on a riboflavin-deficient diet from 40 days before pregnancy and throughout the period of pregnancy. Only 30 out of 53 rats were born alive and the progeny showed the following malformations: shortness or absence of limbs, syndactylia, cleft palate, shortness of the mandibula, and protrusion of the tongue. A few mothers died towards the end of the gestation period. Pregnancy and lactation are associated with increased requirements for most nutrients including riboflavin. Further, severe injury, illness or burns may increase riboflavin requirements 5-10 times.

Pantothenic Acid. Le Febvres-Boisselet determined the effect of pantothenic-acid deficiency on the offspring of rats. Malformations occurred in 94% of the offspring of the experimental rats on a synthetic diet deficient in pantothenic acid. The malformations included exencephaly, pseudoencephaly, anophthalmus and cleft palate and haemorrhage of the extremities. The parent rats showed no signs of any of these malformations.

Other Deficiencies. Polman studied congenital malformations in certain areas in Holland. The incidence in 2 groups of families was very high. He was unable to state whether iodine-deficiency played any rôle in the causation of the malformations in the Union of South Africa, as well as in many other countries, many thousands of people have been examined in endemic goitre areas and no evidence of an exceptionally high incidence in foetal deaths and/or malformations has been recorded. However, in Switzerland deaf-mutism and retardation of mental development appear to be prevalent in endemic goitre areas. It is, of course, well known that hypothyroidism (iodine deficiency) is associated with retardation in mental and physical development.

According to Bennetts and Chapman and Smith deficiencies in certain minerals (copper and iodine) may cause congenital abnormalities.

(b) Toxic Agents, Including Maternal Diseases and Conditions Which Induce Anoxia in the Foetus

From the investigations conducted by Ingalls et al. and observations made by Olim and Turner it is clear that any disease or condition which induces a reduced supply of oxygen to the foetus may cause malformations. The type and extent of the deformity depend on the stage of development of the foetus, the period during which the insult acts and the intensity of the action. Any type of deformity can be caused in utero by the action of various insults. Ingalls and his co-workers submitted pregnant mice at various stages of pregnancy to rarefied atmospheres and found that maternal anoxia may result in foetal death or congenital malformations. They say, 'The effect on the conceptus varies with the degree of maternal placental anoxia and the stage of gestation at which the anoxia insult occurs'. They conclude that rapidly differentiating tissues are more vulnerable to anoxia than resting or fully-differentiated cells. The method they prescribe for the evaluation of rôle played by teratogenic agents, the foetal host, and the maternal placenta, in the production of acquired congenital anomalies is of great value.

Olim and Turner describe a case of a young mother with a congenital cardiac defect who gave birth to two anencephalic foetuses. Anencephaly was caused by the low arterial oxygen saturation which interfered with the nutrition of the foetus during the cephalic phase of development. Each of the two infants were born at approximately 6 months' gestation. After correction of the cardiac defect by the Blalock operation there was material improvement in arterial oxygenation, so that the patient could then carry on her normal activities without cyanosis or dyspnoea, and she then gave birth to a normal child.

Mitotic Poisons. The following chemicals are known to disturb processes of mitosis: colchicin, selenium, nitrites, 'miracil' (thioxanthone compound), urethane, trypan blue, members of the quinone group, nitrogen mustards, arsenic, sodium cyanate, potassium sulphocyanate, acridine dyes, benzene, phenols, several steroids, γ-hexachlorocyclohexane, L-amino-acenophthen and derivatives, adrenaline, mustard gas, compounds related to mustard gas, stilbamin derivatives, and phenylcinnamic acid nitrites.

Chemical substances which have produced malformations in animals are colchicin, insulin, selenium, urethane, boracic acid, pilocarpine, mustard gas and trypan blue (Gillman et al., Grebe and Windorfer). Dammers
and Frens describe abnormalities of the penis in young rats resulting from the administration of iodocasein. Steammel states that in animals it has been proved that alcohol causes changes in mutation and damages sperm cells and that we must accept the same possibility in man. Gillman et al found that a single injection of 1 ml of 1% aqueous solution of trypan blue given to female rats during the early stages of pregnancy could result in gross malformations in their offspring whereas an injection given before as well as another during pregnancy greatly enhanced the incidence of gross defects in the new-born. It is of importance to note that congenital malformations are rare in the offspring of mothers given a single injection of trypan blue before pregnancy but that metabolic disorders, such as jaundice, occurred in 1.8% of the newborn.

The following congenital defects in the offspring of mother rats injected with trypan blue were recorded by Gillman and his co-workers: Eye, ear and tail defects, hydrocephalus, hip dislocations, spina bifida, umbilical hernia, cleft palate, harelip, deformed hindlimbs, clubfoot, skull defects, meningocoele, imperforate anus, microcephalus, and cranioschisis. The total percentage of abnormality was 32.

Fraser et al. produced cleft palates in 79% of the offspring of pregnant mother mice injected with cortisone. An important feature of their results is the fact that cleft palate also occurred in foetuses when treatment of the mother mice with cortisone was started after the 12th day of gestation, when the nasomaxillary fissure is said to have closed. There is thus a possibility of cleft palate due to degenerative changes. The quantities of cortisone administered to the pregnant mice were large. Nevertheless, the results are of great significance to man as cortisone is used in treatment on a large and growing scale.

Kimball and Chury refer to the induction of mutations in animals by radiation, while Loveless discusses the quantitative aspects of the chemistry and biology of radiomimetic (mutagenic) substances. Lettré et al. investigated synergists of mitosis. They found that phlorhizin stimulated non-mitotic quantities of colchicine and N-methylcolchicamide into mitotic action. They suggest that a possible explanation of this phenomenon is the inhibition of phosphorylation processes by phlorhizin.

The observations made by Grebe and Windorfer are of very great significance as indicating that chemical abortificents and chemical contraceptives are possible causes of congenital malformations. They describe a case where a mother had given birth to a deformed child after she had used contraceptive tablets. The child showed anophthalmia (left), microphthalmia (right) with defective eye-lid formation, harelip, cleft palate, microcephalus, arhinencephalia (left), absence of nervus and tractus opticus, congenital heart defect with subaortal septum defect and common atrium, stenosis of aorta isthmus, and absence of the anal orifice. It died 3 months after having been admitted to hospital.

Hone and Magarey described a case of cretinism caused by the administration of methythiouracil to the mother during pregnancy. They recommend that in cases where it is necessary to administer this drug during pregnancy, administration should always be discontinued at least 3 weeks before term. Poate's experience has been that if the smallest effective doses of 'thio' drugs in thyrotoxicosis is given, no harm is done to the foetus. He states that 'colloid does not appear in the gland of the human foetus until about the 6th month, so that the possible danger-period to the foetus is from this time until full term, when thyroxine and iodine are necessary'. He rightly recommends that it is wise to stop administration of antithyroid drugs in the last 6 to 10 weeks of pregnancy and to give small doses of Lugol's solution (3 drops twice daily) and to continue this for 3 months after parturition.

Bickenbach et al. administered methylthiouracil orally to pregnant rats. The young of those mothers which received the drug during pregnancy and lactation, or during lactation only, showed all the signs of cretinism. Szontagh and Lichner found that methyl- and propylthiouracil decreased the number of oestrus days in young healthy rats. Histologically the ovaries showed marked luteinization and few follicles.

DISCUSSION

Practical experience and the results of investigations into the effects of vitamin deficiencies, anoxia, maternal diseases and mitotic poisons on animal and human embryos turn our minds to the practical application of the knowledge acquired. Heredity can no longer be regarded as the sole cause of teratism. As a matter of fact, it appears that the majority of foetal deaths and malformations are due to causes other than heredity; consequently efforts on the right lines can and will prevent many of the common congenital malformations. It appears that not only vitamin deficiencies (A, B1, B12, folic acid, riboflavin and pantothenic acid) before and during pregnancy but also conditions (haemorrhage, maternal heart defects, etc.) or poisons which induce anoxia in the foetus, maternal diseases (rubella), or chemical substances which disturb mitotic processes, may cause different types of congenital malformations in man and animal. The nature of the foetal abnormalities depends on (1) the type of insult, (2) the degree of the insult, (3) the length of the period of its action, and (4) the stage of development of the foetus. The younger the foetus and the more active the development of the tissue is at the time the insult acts, the greater and more extensive the abnormalities will be. It should be noted how prevalent harelip and cleft palate are among congenital abnormalities irrespective of the cause of the deformities.

Gillman et al. have shown that we should think not only of physical abnormalities but also of metabolic disturbances (e.g. jaundice) in the foetus as a result of insults during pregnancy.

From the work of Fraser and his associates it appears possible that some agents (cortisone) may not only prevent the full development of certain foetal tissues but may even destroy tissues which have already been formed.

Ingall's publications have thrown much light on the possible in utero causes of mongolism. He suggests that we centre our attention on the 6th to 9th weeks of foetal life in our search for possible etiologic agents (insults). He states, 'While the causative agents of mongolism are relatively numerous, the causative mechanisms are few in number and operate at about the
8th week of fetal life. Causative agents include hemorrhage, threatened abortion, pathologic abnormalities of the uterus and certain acute intercurrent infections. Experimental and clinical evidence suggests that lack of oxygen to the fetus may be an important mechanism, with temporary starvation and the accumulation of toxic metabolites to be evaluated'.

De Rudder 43 states that at present the general view is that the syndrome of mongolism is caused by damage to the plasma of the ovum which was fertilized. He refers to the observation made by Klebanow that in children of mothers who have during pregnancy experienced extreme physical, spiritual and mental stress the percentage of mongolism is very high. According to de Rudder the often suspected hereditary nature of mongolism has not been proved.

In all cases of congenital physical and mental defects due consideration should be given to the possible rôle played by chemical contraceptives, abortificients, and drugs used during pregnancy.

From the literature it is clear that attention should also be paid to synergists of mitotic poisons, for the former enhance the harmful effects of the latter.

REFERENCES


ABSTRACT


The decrease in the death rate during the first year of life has not been accompanied by a corresponding decrease in the rate during the first 24 hours. Only through medical skill will it be possible to further reduce mortality of the newborn.

While prematurity, asphyxia, atelectasis, birth injuries and congenital malformations contribute to neonatal death, other factors increase the chance of survival, e.g. good maternal care, elimination of infections, adequate nutrition, and skilful delivery with a minimum of interference and untoward effects from analgesia and anaesthesia.

To establish extra-uterine respiration, when postural drainage and suction do not suffice, direct laryngoscopy and tracheal suction may save life, and so may mouth-to-mouth insufflation.

Initial examination of the newborn is important, with careful search for bleeding point, jaundice, heart murmurs and lung lesions, and for evidence of congenital malformations or birth trauma.

A thorough examination of the anterior fontanelles should be given particular attention. Further examination should be carried out. Infants born of diabetic mothers who have during pregnancy experienced extreme physical, spiritual and mental stress should encourage X-ray studies.

Careful search for bleeding should be sought. Disparity in respiratory excursion and cardiac shift should encourage X-ray studies. If anaemia, jaundice or infection is present, haematologic studies should be carried out. Infants born of diabetic mothers and those delivered post-maturely or by Caesarean section should be given particular attention.