CENTRAL INSTITUTE OF ORTHOPAEDICS, SAFDARJANG HOSPITAL, NEW DELHI-16, INDIA

SIGNIFICANCE OF EXPERIMENTAL TERATOGENESIS IN ORTHOPAEDIC SURGERY

By

P. K. DURAISWAMI
Liverpool

During the nineteenth century a new line of research was conducted by great pioneers such as Saint-Hilaire, Liharzik, Valentin, Gerlach, Lombardini and Dareste to establish on a scientific basis the department of experimental pathology known as "*teratogenesis* or the artificial production of malformations and monstrosities". A detailed review of the work done on experimental teratogenesis by various researchers has been given by the author in one of his previous articles (*Duraiswami* 1952). The author became interested in experimental teratogenesis as a result of an accidental observation he had made in 1943 while investigating the rôle of glycogen granules in hypertrophic cartilage cells in chick embryos during endochondral ossification (*Harris* 1932). In the course of his first series of experiments he injected five units of insulin into hens' eggs at various intervals after the beginning of incubation and, to his pleasant surprise, he noticed that some of the chicks showed radiographic appearances resembling osteogenesis imperfecta in the human. Some of them had shown abnormalities such as spina bifida, partial or complete suppression of development of one or more vertebrae, while others showed various deformities in the limbs. Since then a variety of congenital defects have been induced with sulphonamide compounds, thallium salts, cortisone, 3-acetylpyridine, lead salts, benzyl alcohol, isonicotinic acid hydrazide, and certain antibiotics such as synermycin, terramycin and cycloserine, (*Duraiswami* 1952, 1954, 1955, 1959, 1961).

MATERIAL AND METHODS

The experiments were conducted with eggs from white leg-horn, New Hampshire red and Rhode Island Red chickens which were known to be free from genetic

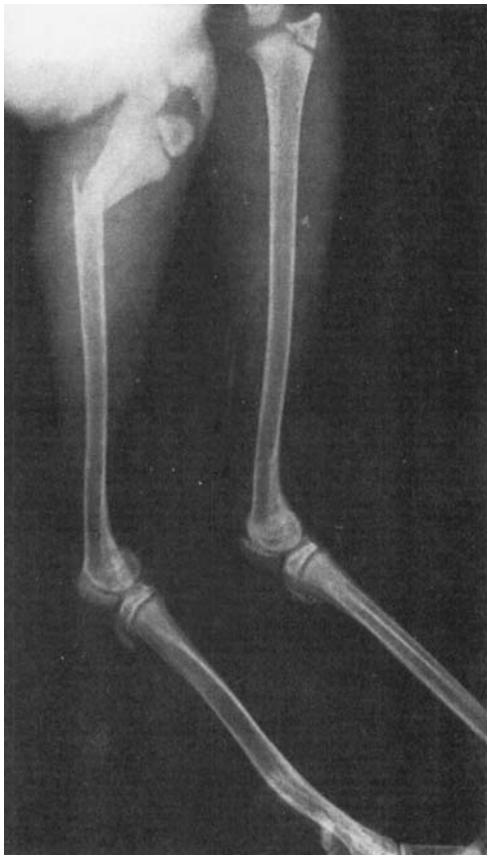

Fig. 1.
Radiograph of a pathological fracture of the left tibia in a 5-month-old chicken with insulin-induced osteogenesis imperfecta (resembling "postnatal" type). The left first metatarsal shows mal-united pathological fracture which preceded the tibial fracture by about two months. Right tibia and right first metatarsal for comparison.

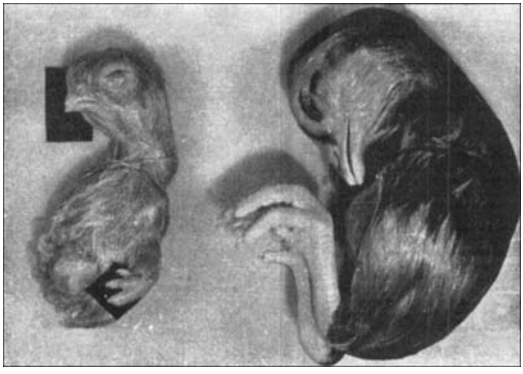

Fig. 2.
Twenty-day-old embryo showing very short limbs ('micromelia') induced by insulin. Normal twenty-day-old embryo on the right for comparison.

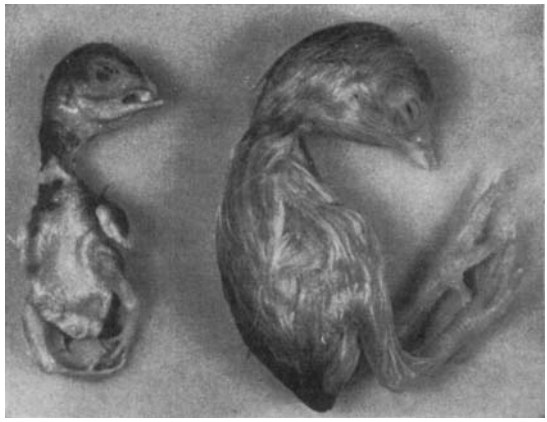

Fig. 3.
Eighteen-day-old embryo showing defects of the limbs, feet, and beak induced by isonicotinic acid hydrazide. Normal eighteen-day-old embryo on the left.

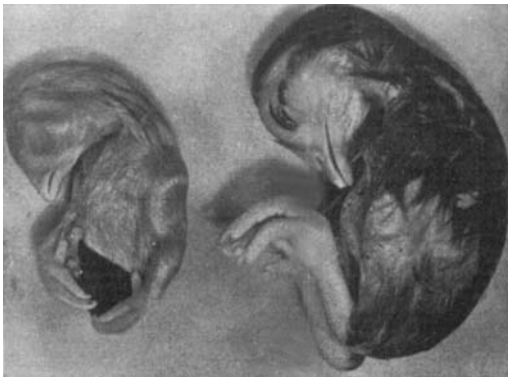

Fig. 4.
Twenty-day-old embryo showing limb and feet deformities induced by synermycin (Pfizer). Normal twenty-day-old embryo on the right.

abnormalities, as well as nutritional deficiencies. The methods employed for incubation of the eggs and for conducting histological and radiological investigations on chick embryos have been described previously (*Duraiswami* 1950, 1952). Some of the defects induced by various teratogenic substances have been illustrated in Figures 1 to 4.

DISCUSSION

In his previous publications (*Duraiswami* 1950, 1952) the author furnished experimental evidence to prove that mechanical trauma of injection was not a factor in causing specific deformities induced by each teratogenic substance, depending on its dose and the time at which it is injected into the egg after the beginning of its incubation. It was also demonstrated (*Duraiswami* 1955) that teratogenic substances such as insulin, cortone acetate, 3-acetylpyridine, and lead nitrate can induce

different types of malformations, though the technique and time of injection, and the method of incubation of eggs are, for alle practical purposes, the same. On the other hand, a limb deformity like the angulation of the tibia leading to pseudarthrosis, can be induced in chick embryos by substances with different chemical compositions, such as insulin, sulphonamide compounds, thallium nitrate, benzyl alcohol, iso-nicotinic acid hydrazide and antibiotics like synermycin, terramycin and cycloserine. It does not necessarily follow that all these substances act in an "unspecific" manner in causing angulation of the tibia. As a matter of fact, the experimental evidence at our disposal points to the contrary.

In the case of insulin it has been observed that hypoglycaemia and profound disturbance to the mucopolysaccharide metabolism may play a part in the causation of the appropriate deformity. On the other hand, sulphonamide compounds seem to induce the defect without lowering the blood sugar of the treated embryos. However, nicotinamide can prevent to a remarkable extent the defects induced by both insulin and sulphonamide compounds. It has been shown by various workers that the developing chick embryos depend entirely on *de novo* formation of nicotinic acid, nicotinamide and phosphopyridine nucleotides. There is adequate experimental evidence to demonstrate that compounds which are chemically unrelated to one another can disturb the normal development of the chick embryos by interfering with the utilization of one or more of these compounds. It is understandable that those parts of the embryo which are in most urgent need of nicotinamide at the time of injection are the ones which are most susceptible.

The question has been raised by some critics whether experimental observations made in lower animals can have any practical application in so far as the etiology and pathology of human congenital defects are concerned. In a previous article *(Duraiswami* 1952) after discussing the various theories regarding the etiology of *human* congenital malformations, the author put forward a hypothesis on the basis of his own experimental work as well as the observations made in experimental teratogenesis by other workers. It is proposed to give a brief summary of the hypothesis and then show its clinical application, as evidenced by various articles published more recently.

The development of an embryo, which is presumably guided by a succession of organizing processes, may be interfered with during "critical periods" by genetic or environmental teratogenic factors. These disturbances to the organizer system may, in their turn, produce

metabolic, biochemical and other changes through the intervention of hormones and enzyme systems and thus interfere with the normal development of the embryo. The resulting anomalies in development would not occur in a random assortment, but tend to fall into certain categories corresponding to the critical stages of development of susceptible tissues and the quality and intensity of the noxious agent. Developmental abnormalities may result not only from arrest of growth and differentiation of the embryo as a whole or some of its parts, as was pointed out by *Stockard* (1920), but also from degeneration in tissues which had developed normally up to a certain stage, as has been clearly demonstrated by the author in the case of insulin-induced deformities (*Duraiswami*, 1950, 1952) and rubella-induced lenticular lesions (*Tondury* 1951).

Now let us see how this general hypothesis can be applied to congenital orthopaedic deformities. In the life of the human embryo it is possible to demarcate certain "critical periods" which are "peculiarly associated with catastrophic changes in the development of the skeleton". (*Harris* 1933). The fourth and fifth weeks of intra-uterine life are definitely associated with the development of the cartilage skeleton. The seventh and ninth weeks present widespread calcification of the cartilage of the long bones as the main feature. The orderly progression from the mesenhymatous condensation to cartilage, and then through calcified cartilage to bone may be disturbed by genetic or environmental teratogenic factors as explained above, and thus may result a variety of single and multiple skeletal deformities. For example, a suppression of proliferation of cells of the mesenchyme which is destined to form a limb will result in phocomelia (total absence of a limb) or ectromelia (absence of a part of a limb). If mitosis in the cartilage is suppressed during the fifth week of intra-uterine life there may be a complete failure of development of the cartilage resulting in agenesis of any of the long bones such as the radius or the fibula, or agenesis of the sacrum and coccyx. In case there is inhibition of mitosis in the cartilage at one end of the bone there will be partial aplasia of the long bone such as the femur or the fibula. Lastly, varying degrees of interference with the nutrition or metabolic processes of the proliferating cells of the mesenchyme, pre-cartilage or cartilage, or any disturbance to endochondral ossification may lead to a variety of abnormalities in a manner broadly analogous to the deformities induced experimentally with insulin and other teratogenic agents.

The large variety of congenital orthopaedic and other defects ob-

served more recently in the offspring of mothers who had taken varying doses of thalidomide ('Distaval' 'Contergen', 'Tensival', 'Valgraine', 'Asmaval' etc.) in early pregnancy appear to the best clinical examples to prove the above hypothesis. The congenital orthopaedic defects observed so far included ectromelia and phocomelia, aplasia of the radius, thumbs and fingers, and major defects of the long bones. The associated defects were atresia, stenosis and malrotation of the gut, absence of the appendix or gall bladder; dysplasias of the external ears or eyes and defects in the heart or genito-urinary system. "Babies with these malformations were born in the Federal German Republic, East Germany, Sweden, Belgium, Switzerland, Australia and the United Kingdom" ("Thalidomide and Congenital Malformations" – Editorial – The Lancet, February 10, 1962, page 307). The actual number of such babies during the past few years is still unknown. However, *Lenz* (1962 (a))has ventured an estimate of at least 2000 and possibly more than 3000 in West Germany alone since 1959. In February 1962 Lenz stated (*Lenz* (1962 (b)) that he was then receiving information on from 3 to 10 new cases daily. It is important to note that thalidomide which is innocuous to the mother like German measles may disturb the growth of limb buds and other tissues in the developing embryo during "critical periods" of their development. According to *Robertson* (1962) thalidomide may seriously interfere with vitamin metabolism, the B-complex especially.

After going through the latest literature on the teratogenic properties of thalidomide, the author is struck with the close similarity between the major limb defects induced in the offspring of mothers treated with thalidomide in early pregnancy and those in chick embryos by insulin. The similarity goes further in as much as insulin-induced developmental defects in chick embryos can be prevented to a remarkable extent by treatment with suitable doses of nicotinamide after injection of insulin and the toxic effects of thalidomide such as glossitis with denudation of the mucous membrane seen in 25 per cent of the cases treated with the drug, as well as polyneuropathy were due to vitamin B-deficiency and these were readily controlled with prophylactic treatment with vitamin B complex. On the basis of this clinical observation one is tempted to suggest that in the case of thalidomide also diphosphopyridine nucleotide-mediated metabolic pathways may be involved in the origin of the congenital malformations and investigations on these lines may be rewarding.

During the past three decades the rôle of maternal diabetes mellitus

in teratogenesis has been studied. In the pre-insulin era, diabetic women had amennorrhoea and were frequently sterile. *White* and coworkers (1939) showed that with the introduction of insulin the fertility of diabetics improved and the maternal mortality dropped to levels comparable with non-diabetic women. However, the early and late foetal deaths fell only from 44 to 38 per cent and there is very definitely a higher incidence of congenital abnormalities. *Oakley & Peel* (1949) reviewed 142 cases examined at King's College Hospital, London and found that the incidence of congenital anomalies was 6.3 per cent as compared with a general incidence of 0.94 per cent in 4829 deliveries in normal mothers. They noted the following congenital defects in their series: internal hydrocephalus, congenital heart defects, hemi-vertebrae, imperforate anus, congenital dislocation of the hip. cleft palate, renal tract malformations, absence of sacrum and coccyx, and talipes equinovarus.

More recently, *Nassim & Jackson-Burrows* (1959) have reported that a six-month stillborn foetus whose mother had suffered from severe diabetes mellitus necessitating large doses of insulin during pregnancy showed multiple congenital deformities of the skeleton, including abnormalities of the vertebral column and commented, "*Duraiswami's*" observations may have a clinical application in pregnancies of diabetic women receiving large doses of insulin ..... The work of *Duraiswami* (1952) is of particular interest to orthopaedic surgeons. By injection of a single dose of insulin into fertilized hens' eggs at an appropriate stage of development he was able to produce a great variety of malformations of the skeleton. The ground substance of bone, being composed in considerable part of mucopolysaccharides, is profoundly disturbed in its metabolism during the period of action of insulin. During that time a fault in the laying down of the skeleton occurs which is never corrected. The moment of injection of insulin in terms of foetal development determines the part of the skeleton in which the defect will appear. The action of insulin is most marked in areas of greatest skeletal activity".

During the past decade great interest has been evinced by researchers in different parts of the world not only in inducing a variety of congenital defects in experimental animals including mammals by introducing chemical and hormonal teratogenic agents into the environment of the developing embryo, but also in making successful investigations with a view to explain the modes of action of at least some of these agents in terms of disturbances to carbohydrate or protein meta-

bolism or to specific biochemical processes. If we can definitely establish the causal relationship between such disturbances and the experimentally induced malformations, as the author is attempting to do in his present series of experiments with certain teratogenic agents, we will have found a way of investigating whether analogous changes in the metabolic, biochemical and other processes are responsible for congenital defects in man also. In this connection it is pertinent to note that *Villee* (1953) has studied the activity of various enzymes by incubating different tissues taken from twenty-six human foetuses obtained at therapeutic interruption of pregnancy in a variety of substrates labelled with radioactive carbon. He is, in fact, trying to describe in biochemical terms the development of the various organs of the human being in the same way as the classical embryologist has described their anatomical differentiation. Work of this nature will go a long way to enable us to explain the etiology of human congenital anomalies on a scientific basis in terms of specific disturbances to normal metabolic and biochemical processes of the developing embryos during the "critical periods" of susceptible tissues and organs, caused by abnormal genes or environmental teratogenic factors. This will naturally pave the way for preventing at least some of the congenital defects in man in the same way as many of the developmental anomalies have been prevented in experimental animals.

SUMMARY

During the nineteenth century a new branch of experimental pathology known as teratogenesis or artificial production of malformations or monstrosities came into vogue mainly through the efforts of Geoffrey Saint-Hilaire and Dareste. Some of the congenital defects induced in chick embryos by insulin, iso-nicotinic acid hydrazide and synermycin have been illustrated. The mode of teratogenic action of insulin has been discussed and its clinical application in the case of the congenital abnormalities induced in the offspring of mothers who had taken thalidomide in early pregnancy has been suggested.

RESUME

Au 19ème siècle une nouvelle branche de pathologie expérimentale connue sous le nom de tératogenèse ou production artificielle de malformations ou demonstruosités a été à la mode principalement en raison

des travaux de Geoffrey Saint-Hilaire et de Dareste. Certaines des défectuosités congénitales provoquées dans les embryons de poulets par l'introduction d'insuline, d'hydracide iso-nicotinique, de synermycine, terramycine et cyclosérine ont été illustrés. L'action tératogène de l'insuline est discutée et son application clinique dans le cas d'anomalies congénitales chez les enfants de mères qui ont pris de la thalidomide au début de la grossessse est suggérée.

ZUSAMMENFASSUNG

Während des neunzehten Jahrhunderts kam ein neuer Zweig der experimentellen Pathologie, bekannt unter dem Namen Teratogenesis oder künstliche Erzeugung von Missbildungen und Monstrositeten, hauptsächlich infolge der Bestrebungen von Geoffrey Saint-Hilaire und Dareste in Mode. Einige der kongenitalen Defekte, die in Hühnerembrionen mittels Insulin, Isonikotinsäure-Hydracid, Synermycin, Terramycin und Cycloserin hervorgerufen wurden, sind erläutert worden. Die Art der teratogenetischen Wirkung des Insulin wurde besprochen und ihre klinische Anwendung im Falle von kongenitalen Anomalien bei der Nachkommenschaft von Müttern, die in früher Schwangerschaft Thalidomid eingenommen hatten, wurde vorgeschlagen.

REFERENCES

1. *Duraiswami, P. K.:* Insulin-induced skeletal abnormalities in developing chickens. British Medical Journal **2**: 384–390, 1950.
2. *Duraiswami, P. K.:* Experimental causation of congenital skeletal defects and its significance in orthopaedic surgery. Journal of Bone and Joint Surgery *34 B*: 646–698, 1952.
3. *Duraiswami, P. K.:* Experimental teratogenesis with benzyl alcohol. Preliminary report. Bulletin of Johns Hopkins Hospital *95*: 57–67, 1954.
4. *Duraiswami, P. K.:* Comparison of congenital defects induced in developing chickens by certain teratogenic agents with those caused by insulin. Journal of Bone and Joint Surgery *37 A*: 277–294, 1955.
5. *Duraiswami, P. K.:* Experimental causation of congenital defects. Proceedings of the Academy of Medical Sciences I, No. 3, 1959.
6. *Duraiswami, P. K.:* Experimental induction of congenital defects. Proceedings of the Eighth Congress of the International Society of Orthopaedic Surgery and Traumatology held in New York (September 4–10, 1960) (in press).
7. *Duraiswami, P. K.:* Experimental induction of congenital defects with iso-nicotinic acid hydrazide. Indian Journal of Medical Research *49*: 631–634, 1961.
8. *Harris, H. A.:* Glycogen in cartilage. Nature *130:* 501, 1932.

9. *Harris, H. A.:* Bone Growth in Health and Disease. London, Humphrey Milford Oxford University Press. 1933.
10. *Lenz, W.:* Thalidomide and congenital abnormalities. Lancet, January 6, 1962, p. 45, 1962(a).
11. *Lenz, W.:* Thalidomide and congenital abnormalities. Lancet, February 3, 1962, p. 271, 1962(b).
12. *Nassim, R. & Jackson-Burrows, H.:* Modern Trends in Diseases of the Vertebral Column. Butterworth & Co., Ltd., London, 1959, p.p. 30–31.
13. *Oakley, W. & Peel, J.:* Proceedings of the 12th British Congress of Obstetrics and Gynaecology. 1949.
14. *Robertson, W. F.:* Thalidomide ('Distaval') and vitamin B deficiency. British Medical Journal, March 17, 1962, p. 792.
15. *Stockard, C. R.:* Developmental rate and structural expression: an experimental study of twins, "Double monsters", and single deformities and interaction among embryonic organs during their origin and development. American Journal of Anatomy *28*: 115, 1920.
16. *Tondury, G.:* Bulletin der Schweizerischen Akademie der Medizinischen Wissenschaften 7: 325, 1951.
17. *Villee, C. A.:* Biochemical differentiation of human foetal tissues with age. In Prematurity, Congenital Malformation and Birth Injury, p.p. 79–83 (Conference at the New York Academy of Medicine, June, 1952). New York. Association for the Aid of Crippled Children, 1953.
18. *White, P., Titus, R. S., Joslin, E.P. & Hunt, H.:* Prediction and prevention of late pregnancy accidents in diabetes. American Journal of the Medical Sciences *198*: 482, 1939.