REVIEW ARTICLE

Mechanisms of teratogenesis*

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INTRODUCTION

In view of the great number of teratogenic factors known and the vast array of congenital defects, disorders and syndromes, it would probably be a waste of time to search for unifying mechanisms and principles in abnormal development. Instead, therefore, I shall describe a selection of teratogens and their consequences, and try to arrange them in a certain hierarchy based on a simplified model of how they act.

The assumption underlying the model (Figs. 1 and 2) is that the result of a teratogenic insult is determined by its site of action and the stage of development of the target organ. This is supposed to hold for all congenital defects, whether due to genes or caused by exogenous agents. In genetic defects the scheme indicates the site and stage of development at which the mutant gene is expressed; in nongenetic defects the site and stage refer to exposure to an exogenous teratogen.

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The four main levels of action of a defective gene or an exogenous teratogen are illustrated in Fig. 1. Action may primarily be exerted on (1) the intracellular compartment, i.e. on the chain of interactions between the nucleus and the cytoplasm leading to the specific metabolic products of the cell. The primary action may also be expressed as (2) abnormalities in the structure and function of the cell surface, or (3) of the extracellular matrix. Finally, detectable defects may result from primary effects on (4) the fetal environment, i.e. from abnormalities at the organismal level or in the feto-maternal relation. Although such a schematic classification into four main levels of teratogenic action is no doubt an oversimplification, it may be useful to consider the mechanisms involved in abnormal development in such a hierarchy. In what follows, examples of both genetic and nongenetic defects of these four categories of teratogenic insult will be presented.
Intracellular compartment

During the last two decades, the processes of nucleo-cytoplasmic interaction have been mapped, and with this rapidly growing knowledge, information has accumulated on the modes of action of mutant genes and of various inhibitors.

Genetic. Perhaps the best analyzed hereditary defects in the intracellular compartment are the 'inborn errors of metabolism', a category of diseases so named long ago by Sir Archibald Garrod. In these diseases a mutant gene leads to deficiency of an enzyme activity, with the result that a metabolic pathway is blocked. There are three possible consequences, (1) the metabolite before the block may accumulate, (2) the metabolite beyond the deficient enzyme may be lacking, and (3) the metabolite before the block may take an alternative pathway. An example of the first type will illustrate how such an intracellular mishap may profoundly affect the whole organism.

In the mucopolysaccharidoses, enzyme defects result in blockage of the degradation of mucopolysaccharides and accumulation (or secretion) of their polymers. For instance, Hurler's syndrome is due to deficiency of the lysosomal enzyme α-L-iduronidase, and subsequent accumulation of both heparin sulfate and dermatan sulfate in the cells. Excessive accumulation of these metabolites in cells of various types gradually leads to the manifestation of a severe disorder, characterized by retardation of growth, mental regression, and various skeletal defects, accompanied by cardiac failure with hepatosplenomegaly (Leroy & Crocker, 1966).

Nongenetic. The various steps of the nucleocytoplasmic interactions may be blocked more or less specifically by appropriate inhibitors. As, by definition, these inhibitors prevent the process of the reading of genetic information, they should all cause defects mimicking hereditary disorders (phenocopies). However, this may be difficult to demonstrate for two reasons. If the function inhibited is essential to life, the effect is usually lethal and so can never give rise to a detectable malformation (Wilson, 1973). Secondly, the effect may be reversible, and the result therefore transient. The first difficulty has been overcome by treating isolated organs in vitro followed by retransplantation. By this method, actinomycin D has been shown to be teratogenic to early brain anlagen, where it produces severe eye defects (Diethelm & Schowing, 1974). Concerning reversible inhibition, tissue culture methods have proved useful, because normal and disturbed development can be followed constantly. Cycloheximidine, when added to cultures of kidney rudiments, stops both protein synthesis and morphogenesis. But after the drug is withdrawn, both processes continue normally, and the short lag period is masked by the subsequent normal development (Saxén, unpublished observations).
Cell surface

Experiments of various types have repeatedly shown the importance of the cell surface in many developmental processes such as cell recognition and aggregation, morphogenetic cell interactions, and cell migrations (see Moscona, 1974a). Consequently, it may be expected that both genetic defects and experimentally induced alterations of the cell surface will lead to impaired development.

Genetic. Extensive studies on mice with mutant genes at the T locus have shown that the homozygotes have various defects and are usually inviable. One of these lethal alleles, $t^9$, seems to exert its effect via a primary abnormality of the cell surface. Development seems to proceed normally until gastrulation. Then, migration of the mesodermal cells between the ecto- and entoderm is severely impaired, and ectodermal–mesenchymal relations are disrupted. Electron microscopy shows that at the gastrula stage embryos homozygous for the $t^9$ gene have mesodermal cells of abnormal shape and with abnormal intercellular relations. Genes at the T locus also determine surface components on spermatozoa carrying the various mutants. The surface antigens of the four $t$ alleles so far tested are different, and no cross-reactions have been detected. Thus the $t^9$ allele seems to act primarily upon the cell surface components, thereby leading to impaired cell-to-cell interactions and altered cell behavior and ultimately to a total block of development (Bennett, 1975).

Nongenetic. The formation of the secondary palate terminates with a fusion of the palatal shelves, a process which can occur only after the epithelium lining the mesenchymal shelves has disappeared. If this ‘morphogenetic cell death’ were prevented, fusion would be incomplete, and the result would probably be a cleft palate. Death of the epithelial cells and fusion of the shelves were preceded by rapid synthesis of mucopolysaccharides on the epithelial surface. This observation led Pratt and his collaborators (Pratt & Hassell, 1975; Greene & Pratt, 1975) to study the effect of inhibiting polysaccharide synthesis. The antagonist of glutamine, 6-diazo-5-oxo-norleucine (DON), was used to block the synthesis of glycosaminoglycans, and the success of the treatment was confirmed by various techniques of light and electron microscopy. In the presence of DON, epithelial necrosis was prevented, and subsequent fusion of the shelves was incomplete. Excess of glutamine restored the synthesis of the surface-associated glycosaminoglycans and allowed programmed death of the epithelial cells and complete fusion.

Extracellular matrix

In various tissues, structure and function are defined by an extracellular matrix. Each matrix has a composition unique to its particular tissue. Hence a teratogen may be expected to affect the function of a tissue by interfering with the production or maturation of this extracellular material.
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Genetic. Dermatosparaxis is a hereditary disease recently detected in cattle, and characterized by a fragile skin with an inelastic dermal tissue. The basic defect seems to be abnormal or insufficient production of collagen in the dermal connective tissue. The mutant cells are devoid of the enzyme procollagen peptidase. Consequently, the procollagen accumulated is abnormal, containing N-terminal extensions of noncollagen protein and its capacity to form fibrils is defective. This, in turn, leads to a severe disturbance in the architecture of the collagen fibrils and in their cross-linkages (Lenaers, Ansay, Nusgens & Lapière, 1971; Hanset & Lapière, 1974). The same enzyme defect was subsequently found in patients suffering from one form of the hereditary Ehlers–Danlos syndrome (Lichtenstein et al. 1973).

Nongenetic. The antibiotics of the tetracycline group will serve to illustrate how teratogens may affect development by acting on extracellular compounds, and not directly on the cells producing these molecules. The tetracyclines are known to be incorporated in the mineralizing tissues, where they may retard growth and cause hypoplasia. Their mode of action has been thoroughly analyzed in vitro, with fetal mouse bones as target tissue. At concentrations that inhibited bone growth, tetracycline hydrochloride did not reduce the rate of proliferation of chondroblasts. Nor did these concentrations affect the synthesis of collagen and mucopolysaccharides, which are the main components of the cartilaginous matrix. The primary action of the drug was finally shown to be exerted on the bone mineral crystals; in the presence of low concentrations of tetracycline their formation and growth were definitely retarded. The drug apparently acts by competing with the inorganic ions building the bone mineral (Kaitila, 1971; Saxén & Kaitila, 1972).

Fetal environment

The development of a mammalian embryo is controlled by a complex of factors, maternal, placental, and autogenous; these factors include hormones, protective mechanisms (immune system), and nutritional factors. Changes in these might be expected to lead to developmental abnormalities.

Genetic. Numerous hereditary defects give rise to changes in the internal environment that secondarily affect various organs. Many endocrine disorders can be placed in this category. For example, the hereditary Pendred’s syndrome is characterized by congenital deafness and hypothyroidism. To study the association between these two defects, Deol (1973) mimicked the syndrome in mice by treating pregnant females with propylthiouracil. This treatment led to severe lesions in the inner ear, especially in the tectorial membrane, which caused loss of hearing. Addition of thyroxine to the mother’s drinking water resulted in offspring with normal morphology of the inner ear and normal hearing. These experimental results suggest that in Pendred’s syndrome, deafness is a secondary consequence of the endocrine disorder.

A peculiar hereditary syndrome in mice is caused by the ‘tail-short’ mutant
gene. This mutant is characterized by a short, kinky tail associated with various other skeletal defects and retarded growth. Deol (1961) showed that in the early stages of development affected embryos suffer from transient anemia. This can be traced back to the formation of blood in the yolk-sac; at this stage the mutants have smaller blood islets than the wild-type embryos. This led Deol to suggest that anemia, by leading to differential retardation of various organs, might be the primary cause of the syndrome.

_Nongenetic._ The embryonic 'macromilieu' is susceptible to many external influences: nutritional deficiencies, placental insufficiency, altered maternal endocrine status and immunization following feto-maternal incompatibility may have deleterious effects on the developing organism. As an example, I shall cite the work of Brent (summarized in 1971). He showed that rabbit antisera against certain rat tissues, when injected into pregnant rats, caused congenital defects in the offspring. Yet the antibodies never crossed the placenta. They accumulated in the yolk-sac epithelium, a structure peculiar to the placenta in rodents and rabbits. There, apparently, the immunoglobulins impaired placental function by impeding the transport of nutritional and other vital factors. A similar mode of action has been suggested for certain azo dyes that cause specific defects in embryos without actually entering the fetal organism (Beck, 1967).

**STAGE OF ACTION**

*Introduction*

The development of various organs during embryogenesis is best considered not as a continuous process of 'maturation', but as a chain of distinguishable steps strictly controlled in time and space. The fate of cells, which were originally identical with the same genome, diverges step by step, and this determination is followed by various events that lead to an orderly arrangement of tissue components of specific shape, size and function. There is good reason to believe that these basic steps of development are highly sensitive to both hereditary and exogenous factors, and that insults at such stages will have profound teratological consequences. Thus, development involves a series of tight corners at which normal embryogenesis is determined, but vulnerability is increased.

Fig. 2 illustrates the development of a hypothetical organ, in which the following events can be distinguished: **determination, proliferation, cellular organization, migration, and morphogenetic cell death.** Several congenital defects and syndromes, both genetic and nongenetic, can be traced back to failures in these events.

**Determination**

The determination of embryonic cells with the same genetic information and their subsequent morphogenesis are processes guided by the position of the cell and its relation to other cells. Determination thus depends on interaction between cells. Numerous examples of such 'inductive cell interactions' have
been described, but the underlying molecular mechanisms are virtually unknown (see Kratochwil, 1972; Saxén et al., 1976). These events are likewise sensitive to both genetic and exogenous factors.

**Genetic.** A mutant strain of mice in which the homozygotes are born eyeless or with severe microphthalmia was analyzed by Chase & Chase (1941). In homozygous embryos the early development of the optic vesicle was completely normal and progressed as in the wild type. Before the stage at which the optic cup makes contact with the overlying presumptive lens epidermis, growth seemed to slow down and the two tissue components either failed to meet or established contact over a restricted area only. Consequently, the ‘inducing stimulus’ which determines the lens and requires close apposition of the tissues was not passed from the optic bud and no lens developed (or the lens formed was very small). Subsequent studies on this mutant strain have suggested that the primary cause of incomplete interaction might be not the retarded growth of
the optic bud, but failure of some intervening mesenchymal cells to undergo normal 'morphogenetic cell death' (see below) (Silver & Hughes, 1974).

**Nongenetic.** Inductive cell interactions can be blocked by various means, both chemical and physical. The effects of various chemicals are difficult to interpret, because our knowledge of the molecular basis of these interactions is so fragmentary. Like various mutant genes, an inhibitor may act on the inductor tissues, on the transmission of inductive signals, or directly on the responding tissue (see Saxén, 1973). Some direct physical effects are easier to explain. The example deals with one of the best studied interactive systems, that occurring between the amphibian gastrula ectoderm and the invaginating mesoderm of the archenteron roof. In his classic experiment, Mangold (1931) interfered with this process by a microsurgical manipulation. He cut a flap in the responding ectoderm, the presumptive neural plate, lifted it, removed a piece from the midline of the underlying archenteron roof, and replaced the ectoderm in its original position. The wounds healed. The ectoderm was now exposed to an abnormally narrow inductive 'template' of mesoderm. As a result, the neural plate was narrowed and the lateral edges approached each other, the outcome frequently being fused optic cups (synophthalmia). The extreme form was complete fusion of the eyes (cyclopia).

### Proliferation

As spatially and temporally controlled proliferation of cells is vital for normal embryogenesis, disturbances in proliferation rates should result in a variety of defects. General inhibition would result in abnormally small embryos and more localized effects in organ hypoplasia or – when differential proliferation rates determine the shape of the organ – to misshapen or defective organs.

**Genetic.** The anomaly known as dextrocardia, which is frequently associated with situs inversus, appears to result from the action of an autosomal recessive gene. During early embryonic life, the primitive cardiac loop, instead of bulging to the left, bulges to the right. Normal curvature of the cardiac loop and regional differentiation and shaping of the heart have been shown to result from differential proliferation of the embryonic heart cells. The heart cells display this intrinsic capacity *in vitro* in the absence of any other tissues, thus showing that they are somehow programmed for differential proliferation. Consequently, dextrocardia is the result of a failure or rather a reversal of the normal pattern of proliferation in the embryonic heart (DeHaan, 1967).

**Nongenetic.** Irradiation and radiomimetic drugs acting primarily on dividing cells have a typical stage-dependent spectrum of consequences as the tissues usually affected are those that proliferate most rapidly. This can be shown in the developing eye. During early development, mitoses occur throughout the outer layer of the neural retina, but as cytodifferentiation of the central part advances, proliferation is restricted to a narrow peripheral zone. Consequently, triethylene–melamine (TEM), a radiomimetic drug, has different effects at
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different stages. When applied in early development, it produces cell necrosis and blocks mitoses all over the retina, the result being anophthalmia. At a later stage, treatment affects only the peripheral zone of proliferation and the end-result is less drastic, mainly microphthalmia (see Saxén & Rapola, 1969).

Organization

Determination and differentiation of cells lead to surface specialization, with the result that like cells can recognize each other. They then tend to adhere to each other and form aggregates. These are the anlagen for various organs and components of organ.

Genetic. The recognition mechanism and adhesive properties of embryonic cells may be genetically defective. This was shown by Ede and his collaborators, who studied the talpid 3 mutant chick characterized by a variety of skeletal defects (Ede & Agerback, 1968; Ede, Bellairs & Bancroft, 1974). The authors used the technique of enzymatic disaggregation and subsequent reaggregation to study the mesenchymal cells of these embryos. Comparison with a wild-type embryo at the same stage showed that reaggregation of the mutant cells was enhanced and the aggregates formed were larger. Ultrastructural studies showed changes in the surface of these cells and their intercellular relations. In all probability, the many defects in this mutant strain are due to altered surface properties of the mesenchymal cells resulting in increased adhesiveness and abnormal intercellular relations.

Nongenetic. The cells of the embryonic neural retina synthesize glutamine synthetase (GS), an enzyme which can be induced with hydrocortisone (Moscona, 1972). This induction depends on homotypic contacts between the retinal cells and their retinotypic organization. In vitro, dispersed cells on monolayers do not respond to hydrocortisone, but when freshly dissociated cells are allowed to reassemble, the clusters can be induced to synthesize GS (Morris & Moscona, 1971). In vivo, the dependence can be demonstrated by treating the embryos with 5-bromodeoxyuridine (BrdU). This thymidine analog is incorporated into DNA and interferes with cell proliferation. When applied to early embryos with mitoses still occurring throughout the neural retina, it disorganizes the tissue and the chaotic cell population that results does not respond to hydrocortisone. When given somewhat later, similar BrdU treatment does not markedly harm the architecture of the neural retina, and, consequently synthesis of GS can be induced with hydrocortisone (Moscona, 1974b).

Migration

During morphogenesis, in addition to moving and aggregating at random, cells and cell clusters undergo oriented movements. Such migrating cells are restricted to certain paths, apparently provided by neighboring cells or their products. Interference with this guiding principle would stop migration or lead to formation of abnormal patterns.
Genetic. The postnatal development of the weaver mutant mouse (wv) is characterized by gradual regression of the cerebellar cortex and consequent motoric dysfunction. Here electron microscopy has revealed a primary failure in the migration pattern of the granular cells. Normally, these cells proliferate in the external granular layer and migrate from there to their adult position in the granular layer. This migration, a prerequisite for the maturation of the cells, is guided by the fibers of the Bergman glial cells, which extend all the way from the external granular layer to the granular layer. In the mutant mice, Bergman cells are mostly lacking, and the granular cells, deprived of their guiding effect, never reach their final position and ultimately degenerate. Similar changes, though less marked, have been demonstrated in the +/wv heterozygote mice (Rakic & Sidman, 1973; Sidman, 1974).

Nongenetic. The vertebrate heart develops from two lateral populations of precardiac mesenchymal cells. These migrate towards their final anterior and ventral position, and again, this migration of cells and cell clusters seems to be guided. The filamentous processes of the mesenchymal cells sense a path provided by the underlying entoderm. If the binding Ca$^{2+}$ ions are removed from the milieu experimentally, contact between the filaments and their substances is broken, migration stops, and the cells form a paired heart anlage (cardia bifida). The key role of the Ca$^{2+}$ ions was further demonstrated in experiments in which the chelating agent added to the medium was accompanied by an equimolar amount of Ca$^{2+}$. Then migration and heart formation proceeded normally (DeHaan, 1958).

Morphogenetic cell death

Various embryonic organs and tissue components are eliminated selectively during embryogenesis, and this programmed ‘morphogenetic cell death’ can be regarded as one important event in morphogenesis, shaping and carving different organs. Both excessive and inhibited cell death should therefore result in abnormal development and congenital anomalies.

Genetic. Various sites and stages of limb development are characterized by cell death. This is strictly timed and localized. At the borders of the limb-bud, necrotic zones determine the size and shape of the limb, and necrosis between the anlagen separates the digits. In talpid$^3$ mutant chick embryos, these necrotic processes are impai red. Absence of necrotic zones at the limb-bud borders leads to abnormally large limb-buds with supernumerary digits, and incomplete interdigital necrosis results in partial soft-part syndactyly, in which the digits are webbed (Hinchliffe & Ede, 1967; Hinchliffe & Thorogood, 1974).

Nongenetic. Exogenous factors will also prevent interdigital necrosis in normal, wild-type embryos. Chick embryos develop soft-part syndactyly when treated with Janus green before interdigital necrosis is visible (Menkes & Delanu, 1964; Saunders & Fallon, 1966). The experiment has been repeated in rabbit embryos with the same result (Saxén, unpublished observations).
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REMARKS

The schemes of Figs. 1 and 2 postulate that the effect of a teratogen and the pathogenesis of the resulting maldevelopment are determined by the site of action of the teratogen and the stage of development of the target tissue. Various examples from genetics, human pathology and experimental teratology seem to support this classification, but do not necessarily establish its validity. Like all classifications, the scheme should be regarded mainly as an attempt to produce order out of chaos. It is intended as an operational guide until a deeper knowledge of teratogenesis yields a simpler, and, perhaps, more unifying theory of abnormal development.

REFERENCES


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