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Determination of Cell Development, Differentiation and Growth

A Review

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Introduction

It has become increasingly apparent that the basis for biologic variation in relation to disease states is in large part predetermined from early embryonic stages. Consideration of variations of growth and development must take into account the genetic constitution and early embryonic events of tissue and organ development.

The incidence of congenital malformation has been estimated to be 2 to 3 percent of all liveborn infants and may double by one year [133]. Minor abnormalities are even more common [79]. Furthermore, the large variety of well-defined 'inborn errors of metabolism', as well as the less apparent molecular and chromosomal abnormalities, should no doubt be considered as malformations despite the possible lack of gross somatic aberrations. Low birth weight is a frequent accompaniment of congenital disease as well as a reflection of a faulty intrauterine environment [33]. Disease states associated with retarded growth have been discussed recently [22]. This review will summarize some aspects of the recent literature related to embryonic and fetal development with particular emphasis upon the mechanisms for developmental variation.

Despite an intense interest in the etiology of lethal or viable malformations in the fetus and newborn and in the misdirection of metabolic pathways in tissue components from infants and children, basic research must resort to the use of experimental models involving embryonic tissues from such species as the sea urchin, chick, pig or rodent. Hence, the interpretations of the subject matter of this review must take into account species variation as well as the experimental alterations of natural organ or cellular environment.

Growth may be defined as an increase in mass of either a cell, tissue or organ and is the result of protoplasmic synthesis, water uptake, or, in the case of intact tissue, intercellular deposition. It may include *proliferation* or the multiplication of identical cells and may be accompanied by *differentiation* which implies anatomical as well as functional changes. The number of cellular units in a tissue may be related to the deoxyribonucleic acid (DNA) content or nucleocytoplasmic units [24, 59, 143]. Differentiation may refer to physical and chemical organization of subcellular components or to changes in the structure and organization of cells leading to specialized organs.

Control of Embryonic Chemical Development

Protein syntheses during oogenesis and embryogenesis are guided by nuclear and nucleolar ribonucleic acid (RNA) which are in turn controlled by primer DNA. (See appendix for brief summary of currently accepted schema of protein synthetic mechanisms.) DNA is present in the nucleus of the unfertilized sea urchin egg but its behavior during oogenesis is incompletely understood. Nuclear DNA synthesis does not occur until after fertilization [85]. Cytoplasmic 'DNA-like' material present before and after fertilization probably represents a mixture of DNA and DNA-precursors associated in part with other organelles [47]. Although its origin is not known, it may serve in part to supply precursors for the formation of nuclear DNA. DNA replication itself is initiated shortly after fertilization and is in large part dependent upon prevailing *de novo* pathways which are present in the ovum [47]. No doubt there are innumerable variations in availability of precursors, active enzymes and cytoplasmic control mechanisms from cell to cell at different stages of development. Unified concepts for the control of DNA metabolism in the embryo are unrealistic at this time.

Experiments utilizing mutant enucleated frog eggs [14] as well as chemical 'enucleation' with actinomycin D [53] have suggested that the first parts of embryonic development are independent of nuclear function. In fact, protein synthesis in treated eggs proceeds at the same rate as in the untreated controls indicating the presence of stable m-RNA. Furthermore, eggs can develop to the blastula stage despite enucleation [14], actinomycin [53] or x-ray [92] treatment. However, RNA from unfertilized urchin eggs can stimulate the incorporation of amino acids into proteins by ribosomes from rat liver although ribosomes from unfertilized eggs are not responsive [77]. Thus, templates for early embryonic development, although present prior to fertilization, are inactive by virtue of inhibition of the ribosomes with which they are destined to associate.

Although active protein-synthesizing polysome aggregates [139] form only after fertilization [84], m-RNA, which assembles ribosome monomer units, is already present in the unfertilized egg. m-RNA [91], ribosomal RNA (r-RNA) [28] and transfer RNA (t-RNA) [38] have been identified prior to fertilization although active synthesis of only r-RNA [18] and m-RNA [140] have been established during early oogenesis. During late oogenesis there is no net synthesis of RNA. Subsequent to fertilization and during early cleavage and blastulation, the RNA synthesized consists of t-RNA and small amounts of m-RNA [18]. Maximal rates of synthesis of these types of RNA, as well as the initiation and peak of synthesis of r-RNA, occur during gastrulation or later [6, 56]. r-RNA synthesis corresponds with the appearance of nucleoli which contain their own DNA and seem to be controlled by cytoplasmic factors [56]. There is evidence which suggests that the maternal messages are held in a 'masked condition' on heavy particles prior to fertilization [120]. Amino acid-activating enzymes are present in the unfertilized egg but their activity increases during blastulation [76]. Similarly, protein synthesis is rapidly increased after fertilization [90] at a time when all of the other necessary factors for *de novo* protein synthesis are rapidly increasing and/or released from inhibition (see above). It has been noted recently that the pattern of protein synthesis prior to gastrulation is different from that arising during later phases [26], an expected observation, since differentiation depends upon differential gene action and protein synthesis.

In contrast with the extensive knowledge of the early chemical development of amphibian eggs, little is known of the replicating and translating mechanisms in the eggs of other vertebrates. The synthesis of DNA has been shown in cleaving bird [98, 115] and mammalian eggs [82]. As may be the case with amphibian embryos, DNA metabolism in other vertebrates may be controlled in part by availability of precursors or by the accumulation of inhibiting concentrations of DNA metabolites [115]. On the other hand, RNA increases most rapidly during cleavage and blastulation [90] and most RNA synthesis, as in amphibian embryos, represents r-RNA [117]. Subsequently, during gastrulation, there appears to be predominance of m-RNA and t-RNA forming systems [117]. Amino acid-activating systems in chick embryos develop shortly before mature ribosomal units appear and remain constant thereafter [126]. There is considerable evidence for differences in the structure, chemical and functional characteristics of the polyribosomes from chick embryos at early cleavage stages in contrast with those from more mature embryos [9, 147].

Investigation of embryonic mitochondrial metabolism is still in the early stage. The specific role of mitochondria in early embryogenesis is not clear, and the variations of enzyme development from one tissue to another have not been defined in detail [13]. It is of interest that mitochondria have many of the ingredients (DNA, RNA, amino acid-activating systems) necessary for protein synthesis [135], and one would expect that interference with any portion of these processes in mitochondria would lead to aberrations in the formation of essential mitochondrial enzymes.

Another early embryonic control system, the subject of considerable interest, is that concerned with controlled degradation of cells. Such a system has been described in embryonic cell cultures of chondrocytes and is called 'inherent obsolescence' [25]. It is possible that lysosomes play a key role in this process. Indeed, acid phosphatase, which is a lysosomal enzyme, has been found at early embryonic stages [105]. Lysosomal hydrolases may have important roles in mullerian duct regression in male embryos [105]. The proteolytic action of spermatozoa upon the protein coat of the unfertilized ovum [29] and the involution of the tail of the metamorphosing tadpole [101] are probably attributable to lysosomal hydrolytic activity.

It seems obvious that the survival and normal maturation of the oocyte and early embryo are dependent upon complex interrelations between the nucleus, nucleolus and the cytoplasm and their component particles. The succeeding sections of this review will discuss those additional external and internal influences upon early embryogenesis which result in biologic variation and aberration. More complete understanding of the chemical mechanisms must await the development of more highly refined techniques and the nonlethal experimental reproduction of hereditable traits.

Induction in Tissue Differentiation

Differentiation of the three basic tissues (ectoderm, mesoderm, endoderm) to form the organs of the intact subject is well known to students of elementary embryology. It is logical to assume that these processes result from a delicately balanced and precisely timed series of anatomical and chemical events. This is called the process of *induction*.

Interactions of various cell groups during the early stages of oogenesis and embryonic development play primary roles in the development and differentiation of tissues [63, 101]. The cells which exert this influence are called the *inductors* and the chemical transmittors produced by the inductor cells are the *evocators*. The ability of the responsive tissue to become differentiated is a result of its *competence*. Studies of these relations have utilized techniques including microtransplantation of embryonic cells, alterations of chemical environments and tissue culture of cells at various stages of differentiation.

Probably the most primitive inductor is the chorda-mesoderm of the amphibian which forms during early gastrulation and induces the early neural tube which in turn forms the nervous system [116]. The chorda-mesoderm can also induce the formation of a secondary embryo when grafted into another embryo at the same stage of development. As a correlate in birds, the role of the primitive streak of the early chick embryo as a primordium vs its role as a primary inducer has recently been considered [119]. This long-standing mystery of classical embryology is still unsettled. Another query of widespread interest has been the control of the formation of the lens (formed from ectoderm). These investigations have uncovered a striking example of the induction phenomenon [101]. Sequentially, the endodermal wall of the future pharynx, the heart mesoderm and finally the retina contribute to this process. Each of these inducers become less effective by virtue of its own differentiation and through alteration of its spatial relationship with the developing lens. The intensity of the induction is related to the degree of summation of effectiveness of the inducers. There is no evidence for a qualitative difference among these three inductor tissues, but the length of time each is in contact with the differentiating ectoderm may be a limiting factor for maximal induction. Although there is probably specificity for a tissue to form a particular organ upon exposure to appropriate inducers (competence) as established by predetermination in the ovum, other portions of early ectoderm infrequently can form a lens. Similarly, there are gradients in the underlying endoderm and mesoderm with regard to inducer capacities. The farther removed the lens ectoderm from the ideal location with respect to the inducers, the less the chance for lens induction. In contrast with the induction of the lens, precise predetermination of the central nervous system necessitates a more central period for chorda-mesoderm induction of brain ectoderm since slight variations in timing and location prevent successful induction [103]. The development of the ear and nose is dependent upon the same inductors as the lens. Manipulation of the overlying ectoderm can reverse the appropriate organ alignment of the nose and ear on the developing head in relation to each other [62].

Tissue culture techniques in which one form of a primitive and incompletely undifferentiated tissue is grown in the presence of other tissues have revealed a broad spectrum of heterotissue interrelations. Thus, both the epithelial cells and mesenchyme of embryonic mouse salivary gland are essential for the production of differentiated salivary tissue [48]. Embryonic spinal cord induces the formation of kidney tubules from kidney mesoderm [51] and the formation of cartilage from somites [50]. As is the case with lens development, the ability of the cartilage-forming somites to respond to the inductor is dependent upon the degree of differentiation of the inductor and upon the predetermined degree of specificity of the somite to form cartilage. It is evident that advancing differentiation of groups of cells exerts a controlling influence upon other tissues to prevent excessive induction. Other examples of heterotissue interaction in organ differentiation are listed in table I.

The ability to induce differentiation of an organ rudiment in tissue cultures while the inducer substance is separated from the rudiment by a highly porous, thin membrane filter has stimulated the study of evocator substances [4, 49]. Evocators may be transmittors of chemical information from one to another tissue. Although the precise nature of the substances involved remains unknown, there is a considerable amount of information which gives clues to their identity. A particularly well-known example of experimental induction involves differentiation of the pancreas [52, 102] which can develop under tissue culture conditions to the extent of exhibiting amylase activity. If the pancreatic rudiment-derived epithelium is removed from contact with the inductor mesenchyme too early, no further differentiation occurs. On the other hand, beyond this critical time, the epithelium can survive and differentiate without further contact with the inductor. In addition, a critical epithelial cell density is necessary for pancreas formation. This is also the case in experiments performed with limb bud mesenchyme [129]. The latter observations emphasize the importance for interactions among identical cells in order to produce a highly differentiated tissue. Exposure of differentiating pancreatic epithelium during the critical period of inductor effectiveness to actinomycin D, which blocks

Table I. Examples of heterotissue interaction in embryonic induction

Inductor tissue	Primordium	Organ	Reference
Chordamesoderm	ectoderm	neural tube	116
Primitive node	definitive streak	neural tube, notochord, somites	119
Pharyngeal endoderm, heart mesoderm, retina	ectoderm	lens, ears, nose	63, 62
Submandibular capsular mesenchyme	submandibular epithelium	salivary gland	48
Dorsal spinal cord	metanephrogenic mesoderm	kidney tubules	51
Spinal cord	somites	cartilage	50
Ridge mesoderm and apical ectoderm	mesoderm	limb bud	8
Collagen producing fibroblast	single embryonic muscle cells	muscle tissue	58
Salivary mesenchyme	epithelial cells from dorsal pancreatic rudiment (gut endoderm)	pancreatic acinar development	52
Capsular mesenchyme	thymus rudiment	thymus lymphoid maturation	3
Cardiac mesenchyme	pharyngeal endoderm	thyroid	32
Cardiac mesenchyme	endoderm	liver	71
Gonadal cortex	germ cells	ovary	144
Gonadal medulla	germ cells	testis	144

DNA-dependent RNA synthesis, prevents zymogen granule formation and amylase activity. This suggests a role for DNA in the induction process. Attempts have been made to identify the nature of the evocator in this system by fractionation of the inductor tissue [102]. No inductor activity was found in an embryo juice ultrafiltrate, adult liver microsomes, embryonic mitochondria or in collagen. Activity was present in the sedimenting fractions containing nuclei, some cell membranes and microsomes. Treatment of active particulate fractions with RNAse and DNAse had no effect. Trypsin treatment inhibits the active particle fraction. On the other hand, pretreatment of the mesenchyme with actinomycin did not diminish the ability to exhibit inductor activity. These studies suggest that the evocator may reside within the cellular matrix or may be associated with the cellular membrane. Since actinomycin was ineffective, one may conclude that the active product may be synthesized by the mesenchyme cells routinely or, at least, that it has a long half-life and is not produced in response to the addition of the target epithelium. The evocator apparently acts upon the genome of the epithelial cell which then synthesizes specific RNA. The elusiveness of more specific identification of evocator substances is due in large part to the difficulty of differentiating between a prime evocator and essential supporting substrates and cofactors which have only a permissive effect.

As is the case with *in vitro* cell studies where one removes cells from their native environment, it is precarious to assume that the physical-chemical interrelations are the same as in the intact organism. Hence, in studying embryonic differentiation, it is also necessary to study the intact developing egg and embryo. This may be done by relating regional and biochemical patterns and gradients to stages of morphological development as have been done with the sea urchin [57, 68] and some vertebrates [31].

In the case of the sea urchin embryo, there are definite gradients of development characterizing ectodermal differentiation (animalization) and endomesodermal differentiation (vegetalization). The interaction of the animal and vegetal regions is supposed to be mediated by diffusing animal and vegetal substances. A considerable amount of evidence has accumulated [68] to support the importance of these gradients during morphogenesis pointing to sequential themes of protein synthesis, mitochondrial enzyme activity, RNA synthesis and activity of the hexose monophosphate shunt. These patterns correlate in time first with predominance of animal and subsequently with vegetal development of the sea urchin.

Whether or not gradients as applied to the embryonic sea urchin apply in the same restrictive sense to vertebrates is a matter of considerable controversy [31] and will not be discussed here. However, the dependence of cellular division upon proper timing during individual developmental periods in vertebrate embryos is well known [30]. Furthermore, there must be a definitive pattern of movement of the early germ

layers in order to prepare the precise interrelations necessary for the processes of induction (see previous discussion). In a series of experiments utilizing explants of early chick embryos *in vitro*, SPRATT [118, 119] has described a center of growth in which cells are rapidly proliferating and from which cells move out radially in a circumferential manner. He attributes the pattern to mechanical and not to biochemical forces in that the morphogenetic cell movements obey principles of fluid flow. He also showed that a physical hindrance to cellular movement would prevent normal architectural alignment of the germ layers. It is obvious that such alterations would hinder cytodifferentiation possibly by interfering with normal induction. It is at this same stage of development that actinomycin, presumably by interfering with the synthesis of DNA-dependent RNA, has a deleterious effect upon the normal development of the embryonic axis [66]. The abnormal embryos produced by exposure to actinomycin had either abnormalities in or absence of the tail, or absence of various portions of the embryonic axis posterior to the 12th somite. The head regions were not affected. Marked decreases in protein nitrogen, DNA and RNA of posterior regions of treated embryos were noted. It is apparent that either the unaffected portions of the embryo have already acquired a stable m-RNA or that the unaffected cells are impermeable to the antibiotic. These experiments are not only of teratologic interest, but provide an example of the importance of 'gradients' in early embryologic development.

The control of embryonic cellular gradients has been elegantly studied by disaggregation of predestined groups of cells or tissue fragments followed by reaggregation amidst cells or tissue fragments from another primordium [127]. This technique allows examination of the omnipotential of preinduced cells from primitive tissues (ectoderm, endoderm, mesoderm) to form the equivalents of normal embryogenesis while under the influence of normal or abnormal intercellular associations and morphologic alignments. The two general conclusions arising from this work are 1. that different cell types of the amphibian embryo, whether present as single cells, cell sheets or globular cell masses, exhibit tissue-specific tendencies of movement within a composite cell aggregate; and 2. that directed movements are followed by the phenomenon of cell-specificity of adhesion. For example, the strong-spreading tendency of ectodermal epidermis provides an environmental influence for the normal inward movement of the cells of the medullary plate to form the neural tube and lumen. In the absence of the epidermis, the medullary plate remains flat (spina bifida). On the other hand, induction may result in the tendency for invagination of ectodermal primordia of systems such as the eye, nose or ear vesicle. In the absence of this inductive influence, this same ectoderm would simply spread. Examination of the properties of intercellular communication by the measurement of electrical conduction of membranes [72] may explain these phenomena[1] and may be related to the thermodynamic explanations for cellular associations and movements [121].

It is difficult to relate a particular congenital anomaly to interference with a specific phase of induction. However, one may propose that the simultaneous occurrence of somatic abnormalities of different organ systems in an embryo may be due to interruption of the normal events of induction or gradient development.

Endocrine Influences on Embryonic and Fetal Development

Neither oogenesis nor early organogenesis is controlled by fetal endocrine function. Extirpation or absence of the pituitary has no marked influence on the early development or growth of birds [83], rodents [132], or man [64]. However, at later stages of fetal development, the experimental removal of the pituitary leads to decreased size [138], immaturity of external appearance [147] and impaired muscle development [74]. Administration of thyroid-blocking agents arrests development of the chick embryo at late fetal stages whereas most mammals, including rodents and man, have normal fetal growth despite administration of propylthiouracil in utero [83].

The critical role of thyroid hormones in the differentiation of the nervous system has been reviewed recently [34]. There is no evidence that thyroid hormone plays any part in the early organization of those centers mediating behavior but its presence is essential for proper development of cortical structure and function during a critical stage of development. The effects of thyroid deficiency in early life upon brain phospholipid composition [26] and mitochondrial support of protein synthesis [44] have been emphasized. It is interesting to note that thyroxine may enhance central nervous system maturation before an effect is produced on overall body metabolic rate [104]. Even growth of an embryo may be enhanced by thyroxine before the metabolic rate is affected or before endogenous thyroid function begins [10]. Stimulation of the proliferation of cortical neurons of offsprings of pregnant rats injected with growth hormone during early stages of pregnancy may indicate an important effect of growth hormone on brain development [148].

The absence of the thyroid gland prevents normal growth of skeletal muscle [107]. Hypophysectomized

[1] Electropotential differences between embryonic cells recently have been demonstrated [109] and may be related to intercellular connections [128].

rats require both thyroxine and growth hormone replacement for optimal muscle protein and collagen formation [108]. The effect of growth hormone on formation of myosin and sarcoplasmic protein is increased by thyroxine, whereas its effect on collagen formation is independent of thyroxine. The muscle from the thyroid deprived or 'hypophysectomized' chick embryo has a decreased protein : nucleocytoplasmic unit ratio indicating primary effects upon the protein content of the cell. Other studies have related the decreased muscle mass in thyroidectomized animals to inhibition of the normal increase in the number of cells [23]. The production of growth hormone by the rapidly growing immature animal must be adequate for the optimal rate of growth since the injection of growth hormone in the normal 12-day-old bird embryo [27] or in the premature human [20] does not result in further increase of body growth.

The effects of adrenal cortical steroids on early development are complex and still mostly unexplored. That cortisone can induce modifications in fetal development is well known [39, 89] but not relevant to its physiologic role in differentiation and growth. Corticoids in the presence of thyroxine reverse the inhibition of the normal appearance of intestinal alkaline phosphatase activity which occurs after hypophysectomy of the chick embryo [86, 134]. Furthermore, the normal increase of enzymatic activity can be advanced 3 or 4 days by the administration of cortisone or ACTH [87]. It is postulated that corticoids elevate intestinal phosphatase activity by activating a 'conversion enzyme' which promotes changes in the molecular form of the phosphatase [88]. It is probable that corticoids exert a considerable influence on the induction and activity of many enzymes in developing tissues.

The effects of steroid hormones on growth of young animals are not well delineated. Apparently the major handicap to growth in adrenalectomized rats is inadequate food intake [97]. In fact, when adequate food is supplied, their growth is greater than in the normal rat. Indeed, adrenalectomy stimulates amino acid incorporation into protein and RNA synthesis of muscle [36] whereas cortisone inhibits protein synthesis in skeletal muscle [145].

Thus, for the most part, studies of the effects of hormones on embryonic and fetal tissues would indicate that normal growth and function of certain specialized cells are dependent upon an intact endocrine system. However, early embryonic differentiation is independent of endocrine function.

Genetics and Organogenesis

It would be inappropriate to continue this discussion without a section devoted to the relations between chromosomes and tissue development and differentiation. It is well known that genetic endowment has profound effects on growth and development of normal children [43]. The frequent association between a variety of chromosomal and somatic abnormalities has unified efforts of clinicians and basic scientists to solve the problem of how the genotypic and phenotypic are interrelated. Recently this topic has been brilliantly discussed [112]. Its relevance to chemical development and inducers is obvious.

It is apparent that there must be a system of checks or reversible repression of nuclear components (genetic information) in order to prevent chaos during early development. The experimental models using transplantation of a cell nucleus to a viable enucleated amphibian egg with subsequent development of the resulting embryo have permitted examination of this problem [55]. The implantation of a normal blastula nucleus will yield many normal embryos whose development proceeds from the phase of the host egg rather than from that of the donor nucleus. The more advanced the developmental stage of the donor from which the nucleus arises, the higher is the incidence of abnormal embryos, the latter being associated with gross chromosomal aberrations [55]. These abnormalities may arise from disproportionate replication of donor DNA whose specific fate is unknown. However, the new message may in turn be transmitted to other implantations and, therefore, can repress normal development. The interruption of amphibian egg development beyond the blastula stage after the injection of nuclear macromolecules from adult liver cells also results in chromosomal defects in the recipient cells [130]. Transplantation of the abnormal nuclei leads to perpetuation of the arrest at the blastula stage and reproduction of abnormal chromosomal patterns. On the other hand, normal embryos may result from the implantation of nuclei from differentiated intestinal epithelium [55], which is certainly dramatic evidence for cytoplasmic repression of the genetic material in the donor nucleus. It is reasonable to relate these observations to the regulation of embryonic organogenesis as well as to the development of enzyme systems. It is apparent that disproportionate replication and/or differential release of information from a genome, either by primary action or by selective repression of specific repressors, results in predominance of development of a specific tissue or organ at an appropriate time during embryogenesis. Normal function of this complex system will result in a normal embryo whereas any qualitative or quantitative breakdown in its sequence

may result in an organ anomaly, deletion of a structural or catalytic protein or production of an abnormal code for a protein.

An example of repression of genetic material in the mammal is the random inactivation of one of the X-chromosomes of the female early in embryonic life [75]. Although this X is being replicated during each cellular division, it transmits no message for the production of proteins. Furthermore, with regard to the normal human female, there is marked variability in the expression of sex-linked traits which may be due to the expression of one allele in some body cells and another allele in others. Hence, female heterozygotes for glucose-6-phosphate dehydrogenase deficiency have one population of normal red cells and another of deficient red cells [124].

Much work in clinical genetics is still in the gross descriptive phase which relates a symptom complex with an aberration in the chromosomal pattern. It is difficult to relate these findings to a chemical basis for abnormal development. It would be necessary to define the role of specific gene loci in the synthesis of replicating systems, in predetermination of the early germ layers, as well as the control of the intricacies of intracellular anatomy and chemical composition. The relations of the genetic code to abnormalities in protein synthesis and enzyme defects in human disease have been discussed elsewhere [5, 123].

Mechanisms of Teratology

Radiation. Ionizing radiation has highly significant effects upon early organ differentiation and growth. Its role in experimental teratogenesis in a variety of animal species has been studied extensively [90, 99, 100, 142, 146] and its relation to the occurrence of human anomalies has been commented upon [133]. Discussion of this subject will be limited to the principle conclusions derived from a variety of observations, the details of which are described in the reviews mentioned above.

It is recognized that radiosensitivity decreases with increasing differentiation of cells. Furthermore, early embryonic stages of development frequently are associated with increased radiosensitivity of cells. Not surprisingly, radiation-induced abnormalities can be transmitted to an embryo developing from irradiated eggs or sperm. Cells are most sensitive to radiation if they have a high reproductive capacity and are in the process of early differentiation or in the transitional stage of maturation. Radiation has profound effects upon the nucleus and probably suppresses mitotic activity in part by chemical changes in the cytoplasm. The larger the nucleus of the cell or the greater the DNA content per chromosome, the greater the radiosensitivity. Indeed it has been suggested that there would be less percentage loss by chromosomal breakage with increased chromosome number.

It is difficult to predict the effects of radiation on the whole organism since the same tissue or cell may have different radiosensitivities when located in different parts of the body. In fact, irradiation can suppress growth of part of an organ while stimulating the growth of another portion. It is possible that differentiation is affected by radiation only when mitotic activity is a prerequisite for differentiation but the limiting factors must be more precise than this. Hence, gradients or developmental patterns may influence teratologic sensitivity. Furthermore, the role of radiation-induced impairment of vascular supply, even during the post-mitotic phases of cell development, may contribute to apparent alteration in tissue responsiveness to radiation.

There are a multitude of effects of radiation upon the chemistry of the cell. These include alterations in structure and biological function of enzymes [1, 114], hormones [94] and DNA [73, 136] by such processes as free radical formation, loss of active sulfhydryl groups, changes in intramolecular aggregation and macromolecular chain breaks with fragment formation. These conditions result in faulty control of energy production and errors in replication, genetic coding and protein synthesis, any or all of which may interfere with normal differentiation and growth.

Immunologic Influences on the Embryo

The embryo and fetus are victims of their environment and may be adversely affected by maternal disease, placental insufficiency or over-crowding of the uterus [33]. The maternal contribution to a variety of cells and proteins in the fetus has evoked considerable interest in the role of the unique immunologic relations which they share in the production of variations in development [11, 17]. Evidence is cited [11] to suggest that the fetus is protected from rejection by the mother because of the poor antigenicity of the trophoblast which itself is a product of the fetus. Furthermore, with increasing parity, the mother develops a transplantation 'tolerance', the origin of which is controversial. This may be an additional factor in protecting the fetus [11].

Abnormalities of cellular differentiation or growth in the fetus attributable directly to the placental transfer of antibodies have been limited. These include: hemolytic disease [149], transient neutropenia [67] or possibly thrombocytopenia [110], thyrotoxicosis due to transplacental passage of long-acting thyroid stimu-

lator [80] and discoid lupus due to transfer of factors causing the lupus cell phenomenon [61]. Maternal thyroid antibodies are not associated with fetal thyroid disease [21]. Furthermore, the mammalian fetus can survive despite the existence in the mother of homograft sensitivity for which the fetus is the specific target [69].

On the other hand, there have been several experimental attempts to induce congenital malformations by the use of specific tissue antigens or antisera [17]. Autoimmunization of female mice with brain antigen causes significant abnormalities in structures related to the central nervous system or the eye [7]. Antiplacental or kidney antisera cause both maternal disease as well as a broad spectrum of fetal malformations of the brain, eyes, limbs, intraperitoneal structures and kidneys [15]. However, at present, it is difficult to resolve whether these antibodies have a primary effect upon the maternal organism, decidual development, or upon the embryo itself.

The removal of the thymus gland from newborn animals results in marked wasting, decrease in lymphocytic population of blood and tissues, as well as inhibition of normal immunologic response [81]. A similar syndrome can be produced by neonatal treatment with adrenal corticosteroids [106]. Hence, immunologic failure prevents normal growth in the neonate. However, despite marked immunologic deficiencies and diminished extrauterine growth, children with thymic abnormalities [96] and almost complete lack of lymphoid tissue [46] have normal birth weights, indicating that immunologic competence is not necessary for intrauterine development.

Virus-Induced Malformations

Viral interference with normal differentiation and growth of embryonic tissues is well known [12, 113]. Viruses from affected human embryonic tissues have been isolated [2, 137]. Furthermore, injections of virus particles into pregnant animals have produced significant embryonic disease or congenital malformations [37, 111].

There are several interesting considerations relating to the etiology of the teratogenic action of viruses. It is not surprising that the susceptibility of embryonic tissues to alteration by a virus diminishes with increasing differentiation. Thus, polyoma virus interferes with the induction of tubulogenesis in the *in vitro* system of dorsal spinal cord plus metanephric mesenchyme, a combination which supports rapid synthesis of viral protein [131]. However, once the tubular epithelial cells are formed, the tubules are unable to synthesize viral protein and are resistant to the virus. The incorporation of the virus particle into the host cell is associated with inhibition of the RNA synthesis by the host genome. The RNA produced has a base composition complimentary to that of the RNA from the virus particles [65]. There is a generalized depression of host protein synthesis and enzymatic activity [60]. Such viruses as measles, yellow fever, Rous sarcoma and herpes simplex cause morphologically detectable abnormalities in chromosomes which may be mimicked by analogues of naturally occurring nucleic acids, the latter also inhibiting DNA synthesis in the host cells [93]. Virus infection of the embryo also may lead to abnormalities in lipid metabolism [54]. The possibility that virus infection may interfere with normal induction processes or intercellular interaction during cellular differentiation by modification of the cell membrane [78] has not been explored.

Hence, it is not difficult to predict that the elucidation of the effects of virus-induced teratogenesis will indicate interference with the processes of induction and the complex interrelations between primordial embryonic cells, their subcellular constituents and metabolic products. Indeed, it is not surprising that the rubella syndrome includes involvement of the heart, eye, brain and ear, the primordia of which have such intimate relations during early embryonic induction [63].

Drug Related Teratogenesis

The external (environmental) factors to which the developing embryo and fetus are exposed logically have profound effects upon tissue differentiation, organogenesis and growth. A broad spectrum of unrelated factors have been found to be teratogenic. The list includes such varied conditions and chemicals as hypovitaminosis or hypervitaminosis, anoxia or hyperbaric oxygen, thyroid-stimulating hormone, insulin, antibiotics, inorganic ions, central nervous system tran quilizers, cancer chemotherapeutic agents, organic dyes, analogues of naturally occurring substances, as well as chemically inert particles. These have been described at length in recent reviews [122, 141] and will not be elaborated upon here.

However, in light of the mechanisms of normal embryogenesis, many of which have been commented upon in this review, it would be well to consider some of the factors which must be taken into account in the chemical pathogenesis of a teratogenic agent. Aside from the roles of maternal detoxification mechanisms, interference with normal maternal metabolic pathways, transplacental passage variations, alterations in blood supply and chemical transformation of the potentially toxic agent, the teratologist must reconsider the

sum of all of the events of embryogenesis. For example, he must apply his knowledge of the biochemical and pharmacologic actions of the teratogen to the metabolism of each of the primitive germ layers in its role as an inductor and as a target tissue. He must relate the resultant maldevelopment to the timing of events which normally lead to formation of those organs. Then he must differentiate between interference at the time of induction and early differentiation in contrast to an antecedent event in presumable inactive cells whose predetermined role is yet to become expressed. This information may be obtainable from such systems as *in vitro* cell cultures with semipermeable membranes or microtransplantation techniques in intact embryos. Furthermore, there is the difficult task of relating the functions of intracellular organelles, their membranes and intercellular communications to the behavior of organized cells or tissues.

The thalidomide problem is an example of some of the complexities involved [16]. The chemical effects of thalidomide are poorly understood; there is some evidence to suggest that the mesonephros may have a temporary role in the induction of the limb mesenchyme along with the more important influence of the overlying ectodermal ridge [70]. Exposure of explants of embryonic chick to thalidomide, though probably a poor subject for the study of the effects of thalidomide as applied to the human [16] mesonephros, resulted in inhibition of chondrogenesis, whereas no effect was seen in explants of limb cartilage [70].

Genetic Aspects of Teratology

Chromosomal abnormalities which are identifiable by standard techniques frequently are associated with congenital anomalies and may fulfill the requirements for being classified as a syndrome. Occasionally, a pattern of inheritance of the abnormal chromosomal karyotype is described [95]. In addition, it is frequently implied that an abnormal phenotype is due to the aberration in the genotype, but it is not possible to prove the specific causal relation. On the other hand, familial tendencies for recurrence of gross congenital anomalies such as cleft palate [40, 42], spina bifida [40, 42] and congenital heart disease [45] usually are not associated with detectable chromosomal defects. It is probably safe to speculate that the basis for some of these defects is related to interference with normal inductor-target cell relations. The specificity of the genetic requirements for the production of complex chemical constituents is best illustrated by the large number of abnormal protein-synthesizing control mechanisms associated with the well-known inherited metabolic defects.

In the context of teratology, genetics may have an important role insofar as predisposition to the effects of a teratogen. Although not well described in human malformations, it is well known that certain mouse mutants are highly susceptible to such agents as cortisone which cause cleft palates [41]. The species specificity to the effect of thalidomide is another example of the role of genetic predisposition in teratology. It is important to be cautious in assigning a causal role to a chromosomal abnormality in the production of a malformation, since the inciting factor may cause somatic malformation [35] in much the same fashion and simultaneously with the production of chromosomal changes.

Appendix

Mechanisms of Protein Synthesis

Protein synthesis takes place on cytoplasmic organelles called ribosomes which consist of RNA (ribosomal-RNA, r-RNA) and protein and are usually found as aggregates called polyribosomes. The amino acid sequence of the protein chain is directed by the sequence of nucleic acids on a nucleic acid chain, messenger-RNA (m-RNA). The composition of m-RNA is complimentary to DNA located in the nucleus. The m-RNA can 'read off' the nucleic acid code through the sequences of triplets of nucleic acid units which code for one or more specific amino acids. During this transcription the amino acids join together to form a polypeptide. The latter process is facilitated by linkage of a specific amino acid with another form of intermediary RNA called transfer-RNA (t-RNA) whose nucleic acids in turn join temporarily with a specific portion of the m-RNA. As the ribosome moves over the m-RNA, the amino acids leave the t-RNA and join the growing polypeptide chain. The sequence of amino acid build-up is begun from the free amino end ($-NH_2$) of the peptide and grows by formation of a peptide bond between the carboxyl group (–COOH) of the growing chain and the amino group of the newest amino acid. The t-RNA is then released from the m-RNA and the growing peptide is ready for another amino acid until the chain is completed, after which it is released from the ribosome. The process of preparation of the amino acid for attachment to the t-RNA, the union of the amino acid-t-RNA complex with m-RNA, and the transfer of the amino acid to the polypeptide take place through a complex series of reactions which require several cofactors, high energy bonds and specific enzymes. The t-RNA and r-RNA are synthesized in the nucleus and nucleolus, respectively.

The antibiotic actinomycin D is useful in studying the mechanisms of protein synthesis by virtue of its ability to inhibit DNA-dependent replication of RNA.

Although this is thought primarily to involve DNA-directed m-RNA synthesis, there is evidence that it may also inhibit the synthesis of other forms of RNA and may even effect ribosomal structure itself. The antibiotic puromycin inhibits protein synthesis through preferential attachment to the growing polypeptide at the carboxyl group and by preventing the transfer of the next amino acid from its t-RNA, thus prematurely terminating the growth of the new protein.

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