The excess material of one atomic type can be made to diffuse to dislocations where it precipitates. The concentration of vacancies in the other atomic sublattice is therefore reduced along with a reduction in concentration of conduction electrons or holes in the semiconductor. The equilibrium concentration of holes in PbTe crystals as a function of precipitation temperature is shown in Fig. 6 (*5*). This method could in principle be pursued to very low charge carrier concentrations but is limited in practice by the increasingly long diffusion times required to reach equilibrium at lower temperatures.

Summary

Thus, formation of intermetallic compounds must satisfy certain geometrical and electrical considerations, including favorable ion-size ratios, electron to atom ratios and electronegativity ratios. There is no sharp boundary between compounds which have metallic characteristics and those which have semiconducting or insulating characteristics. In any real intermetallic compound there is a superposition of the different types of interatomic bonding and the solids generally have appreciable semiconducting character if the dominant bond force is ionic or covalent.

In the case of semiconducting intermetallic compounds, those with strongly covalent bonds tend to conform closely in composition to the simple proportions given by the chemical formula. Compounds with an ionic component in the bonding tend to exist in the solid phase with various concentrations of vacant lattice sites, interstitials or place exchange. These have a profound effect upon the electrical properties of the solid. The equilibrium concentrations of these defects have been discussed for a few representative compounds. In spite of the many intermetallic compounds being prepared today only a few of them have detailed information on phase diagrams in the vicinity of the stoichiometric composition.

Finally, in intermetallic compound semiconductors, the concentration of electrons and holes is often a complicated function of the phase-equilibrium relationships and is highly sensitive to temperature. The means by which changes occur in the solid fortunately depend upon a diffusion process which, at ordinary low temperatures, is excessively slow. Crystals prepared at high temperatures, to a desired composition, can be quenched to low temperatures where their composition is for all practical purposes fixed.

In a few intermetallic compound semiconductors, principally those with highly covalent interatomic forces, the composition is, essentially, fixed at the stoichiometric composition.

References and Notes

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Lethal Genes and Analysis of Differentiation

In higher organisms lethal genes serve as tools for studies of cell differentiation and cell genetics.

Salome Gluecksohn-Waelsch

Among the many problems of genetics untouched by recent spectacular advances in molecular genetics is that of lethal genes. The reasons for this lie primarily in the fact that lethal-gene problems touch upon various areas of genetics all of which are concerned with gene effects on levels more or less removed from that of primary gene action. Among these areas are population genetics, radiation genetics, and developmental and physiological genetics.

The analysis of gene action at the molecular level stops necessarily at a point where the interaction of genes, of gene products, of cells, and of tissues begins—in short, at the phenotype level. It is, on the other hand, precisely at this level that problems of cell, tissue, and organ differentiation make their appearance, and that those phenomena occur which serve to characterize a gene as a lethal. Even further removed from the molecular level of gene action are problems of lethal genes in populations, and of their origin, whether spontaneous or induced—for example, by radiation.

Because of the great significance of lethal genes in populations, because of their importance in studies of radiation genetics, and because of their role in the analysis of development and differentiation of higher organisms, it appears worth while to attempt at this time a re-evaluation of this problem and to relate it to new concepts of genetics.

In this attempt emphasis will be put on the physiological genetics of lethal genes. The significance of lethal genes in populations has been the subject of numerous discussions (*1*); an extensive literature deals with studies of radiation-induced lethals (*2*). These topics are not taken up here. Problems considered in this article include the mode of action of lethal genes, the properties that distinguish them from other mutant genes, the physiology of the effects of lethal genes, and their utilization in analyzing cell, tissue, and organ differentiation in higher organisms.

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Definition of Lethal Genes

To begin with, it is necessary to define and to discuss briefly the concept of lethal genes.

The term *lethal* is applied to those changes in the genetic makeup of an organism which produce effects severe enough to cause death. The nature of such changes may vary widely, from alterations in the number of chromosomes to changes in chromosome structure and to gene mutations. Lethal gene mutations, with which this article is concerned, are distinguished from other gene mutations only by the severity of their effects. When speaking, therefore, of lethal genes we must realize that these do not represent a separate category but are mutations not different in principle from others compatible with life. Consequently, no sharp borderline can be drawn between "viable" and "lethal" genes. Any attempt to distinguish between them must remain arbitrary, and many variables, including those of the environment, determine whether a particular gene is "viable," having effects compatible with life, or "lethal," resulting in the death of the organism that carries it. This relative character of lethal genes clearly illuminates the problem of the roles of nature and nurture in the expression of genetic traits.

In bacteria, a mutation is considered lethal if growth and survival of the mutant organism cannot be achieved by manipulation of the environment. If, on the other hand, it is possible to devise environmental conditions which permit the organism to survive, the mutation becomes "viable" and therefore may be called a "conditional lethal" mutation. This is the case, for example, when a mutation results in inability of the organism to synthesize an essential amino acid. If the organism is grown on a basic minimal medium, such a mutation will have a lethal effect; if, however, the missing amino acid is supplied from the outside—that is, if a nongenetic modification of the environment is produced—the same mutant becomes "viable." Similarly, it is possible to introduce into the genotype a suppressor gene specific for the mutation in question and thus to achieve a genetic modification of the lethal effect.

Similarly, a mutation in a higher organism which makes the organism unable to synthesize a certain indispensable metabolite must be termed "lethal" unless the vital substance is supplied from the outside. If, for example, a mammalian organism were to become unable, as the result of mutation, to synthesize glycine, this amino acid would have to be supplied from an external source, as is necessary, for example, in the case of the growing chick. If, for some reason, this outside supply of glycine failed, certain essential biosynthetic reactions requiring glycine could not be carried out by the mutant organism and death would ensue; the mutation would then have become "lethal." It has been suggested that a mutation which occurred in the course of evolution is responsible for the human requirement for ascorbic acid from the outside; primates and the guinea pig also have this requirement, whereas other animals appear to be able to synthesize this vitamin themselves. This mutation in man might be termed a conditional lethal mutation, since its effects lead to death unless ascorbic acid is provided in the diet.

Examples such as these emphasize the relative nature of most lethal mutations. The lethal or nonlethal character of a mutation is not an absolute and inherent property of the genetic material in question but is determined by the mechanism of the mutational effect, which may or may not permit compensation by other genes or the environment for deficiencies caused by the genetic alteration.

Lethal genes should, therefore, not be considered a separate class of genes with special characteristics inherent in their material makeup. They are distinguished, rather, by effects which are not compatible with life unless compensated for by other genetic or nongenetic factors. These lethal effects may differ only quantitatively from the effects of other detrimental genes and thus may represent the most extreme expressions of a wide range of mutational effects. Or, the lethal effect may differ qualitatively from the effect of other mutant genes by interfering with a function absolutely vital to the organism.

For purposes of illustration, several series of multiple alleles in the house mouse may be cited; some of the alleles are compatible with the survival of the organism carrying them in single or double dose, while others, in the homozygous condition, cause death. In the *W*-series of multiple alleles in the mouse, which affect the erythropoietic system, viable and lethal alleles seem to be distinguished by quantitative differences of their effects. In another multiple allelic series, that at the so-called agouti locus, the effects of all the alleles but one are compatible with life and relate to pigment formation only. The one lethal allele, yellow, appears to have, in the homozygous condition, an effect qualitatively different from that of the other alleles: it interferes with a function vital to implantation in the uterus and thus causes the death of the embryo. In this case, as well as in other cases, the distinction between qualitative and quantitative differences of allelic effects on the phenotype level is of course not always obvious or actually proved. This is particularly true, for example, in the *T*-series in the mouse, discussed later in some detail.

It may be argued that there exist genetic loci which are "immune" to the appearance of lethal mutations—that is, where no such mutations can be expected to occur. If, for example, the complete lack of a given locus as a result of a chromosomal deficiency is compatible with life, one would perhaps not expect a mutational change at that locus to have lethal effects, since, it might be argued, no change could be more severe than total loss of the genetic region. However, in terms of physiological function, total lack of a metabolite, as the result of the genetic deficiency, could conceivably be more easily compensated for than could the production of an abnormal metabolite by a mutated gene which might result, for example, in a chain of inhibitory reactions.

In the field of population genetics those genes are considered "lethal" which do not contribute to the gene pool of the population; the main concern is not necessarily with the lethal gene's genetic or physiological mechanisms. Consequently, the term *lethal* is applied to those factors which either cause death before the organism is able to reproduce or which interfere in some way with reproductive activity, thus obstructing the transmission of the gene to the next generation.

The term *lethal gene,* then, as used in various areas of genetics, is so loose as to make it appear futile to attempt a definition which would fit all types of "lethal" genes, even approximately.

In view of the many and varied viewpoints from which the problem of lethal genes may be considered, it seems permissible to limit the discussion deliberately, unless a full treatise on lethal genes is planned, such as the exemplary one by Hadorn (*3*). In this

article I use the term *lethal genes* to mean genes that have lethal action at the phenotype level—action which does not necessarily indicate the nature of the change at the gene level and which is subject to the effects of other genetic factors and of nongenetic factors.

The physiological geneticist may have manifold reasons for being interested in lethal genes; the ultimate goal, however, is to learn their mode of action. In the course of such studies, lethal genes become tools for investigating special problems within the framework of interest of the researcher.

In many subdivisions of genetics (such as population, radiation, developmental, and cell genetics, to name just a few), and in other areas of biology, lethal genes have been found to be a rich source of experimental material. In the field of experimental embryology, for example, the causal analysis of developmental mechanisms, or knowledge of cell and tissue differentiation, particularly of higher organisms, has benefited and will continue to benefit from studies of the physiological genetics of lethal genes.

In the discussion that follows, particular emphasis is placed on lethal genes as tools for analyzing the development and differentiation of mammalian embryos and for studying the genetic control of these processes. The utilization of developmental abnormalities for analyzing genetic phenomena goes back to Boveri, who in his classical experimental studies of sea urchin development, around the turn of the century, established the individuality of the chromosomes and their essential role in development. Lethal genes have been shown to produce a wide variety of morphological, physiological, biochemical, and metabolic abnormalities which may lead to the death of the organism before or after birth—or even as late as maturity, thus not necessarily interfering completely with reproductive activity. Some of the mechanisms by which these lethal genes exert their effects are examined here, and possible experimental approaches to their analysis are discussed.

Origin and Frequency of Lethal Genes

Lethal genes may originate in spontaneous mutations or they may be produced by mutagenic agents—for example, radiation or chemicals. The frequency of spontaneous lethal or sublethal recessive mutations has been measured in different species of *Drosophila:* 41.2 percent of chromosomes No. 2 and 32.1 percent of chromosomes No. 3 of South American populations of *Drosophila willistoni* were found to carry them (*4*). The frequencies of such mutations cannot be ascertained easily in man or even in experimental mammals. They have been estimated, however, and according to one such estimate (*5*), a normal person is heterozygous for an average of eight recessive lethal or sublethal genes.

For many reasons, some of them obvious, the chance of discovering recessive lethal genes is much smaller than that of discovering nonlethal mutations (*6*). Nevertheless, studies, by Muller, of sex-linked lethal mutations in *Drosophila* revealed a large number of such mutations—a finding which led him to conclude that, in view of the great methodological difficulties of demonstrating lethals, "probably the majority, if not the vast majority, of mutants are lethals." These studies provided the experimental evidence for Muller's earlier prediction on theoretical grounds that most newly arising mutations would turn out to be lethal. Since, in the course of evolution, Muller argues, the organism had made use of all newly arising mutant factors to build up the best-adapted biological machinery, any new mutant change would be likely to lead to a severe disturbance of this complex mechanism and would thus be "lethal."

The approximate ratio of lethal to visible and viable mutations has been calculated by various workers. In spite of variations in different species, the frequency of lethal factors appears always to be several times that of visible mutations, among spontaneous and induced mutations alike.

Numerically, therefore, lethal genes make up a large portion of the genetic load of a population, which is defined by Crow (*7*) as "the proportion by which the population fitness (or whatever other trait is being considered) is decreased in comparison with an optimum genotype."

Dominant lethal genes come to the attention of the investigator only rarely under normal conditions, and they are eliminated from the population almost as soon as they appear; no studies have been made of their mode of action in higher organisms. The lethal genes considered here are all recessives.

In the course of analyzing the effects of lethal genes, inquiry must be made into the levels at which lethal gene action occurs. Although lethal gene action resides, of course, ultimately in an error on the molecular level, its expression may occur at any of various levels—the level of the cell, the tissue, the organ, or the organism.

The first approach to the study of lethal genes in higher organisms is necessarily descriptive, involving use of the methods of morphology, embryology, physiology, and biochemistry. In this way the foundation may be laid for subsequent experimental probing into causal mechanisms.

Frequently a lethal effect is the end result of a long chain of processes started off by one primary gene-controlled abnormality. In such a case, the search for this primary abnormality may reveal hitherto unknown links in the chain of normal processes, as well as interrelationships between different processes involved in the development and differentiation of a tissue or organ, and may thus contribute to the analysis of pleiotropy—that is, multiple effects of a gene. The phenomenon of pleiotropy in higher organisms has been the cause of many arguments, particularly in respect to its relevance to the analysis of primary gene action, and here reference is made only to several recent discussions (*8, 9*).

Lethal Genes and Organ Differentiation

The lethal effects of genes trace back to widely divergent causal factors and operate through equally divergent mechanisms. A relatively simple situation, from the point of view of analysis, exists when a mutation results in the suppression of normal development of a vital organ and in this way causes death of the organism. The causal analysis of such gene-controlled interference has thrown light on causal mechanisms of normal organ development.

An example of a lethal gene that produces its effect by suppressing differentiation of a normal organ is a semidominant mutation in the mouse: animals homozygous for the gene Sd are completely tailless and die soon after birth because of an almost total lack of kidneys (Fig. 1). Investigation of the steps by which the presence of this lethal gene in homozygous condition leads to the absence of kidneys has contributed to the causal analysis of kidney development in mammals (see *10*).

The descriptive study of abnormal

kidney development in this mouse mutant could do no more than lead to the proposal of certain alternatives as causal mechanisms possibly responsible for the abnormal kidney differentiation. In one of these proposals it was postulated that, as a result of the mutation, the development of the ureter was suppressed and, consequently, the ureter could not exert the necessary inductive effect on the kidney mesenchyme and further differentiation failed to take place. However, a decision between this and other possible interpretations of the observational results could only be made through experimental approaches. Among these, tissue- and organ-culture methods seemed to be best suited for this particular study and were applied in the analysis of kidney differentiation in the mutant embryos (*11*).

To begin with, the developmental potencies of entire kidney rudiments from embryos homozygous for this lethal gene were tested in organ tissue culture. Contrary to the findings for the whole embryo in which kidney differentiation is suppressed, kidney tubule differentiation occurred in the intact "lethal" rudiments in vitro, although to a lesser degree than normal. The question tested next was: To what extent is the suppression of kidney differentiation in the mutant due to interference of the lethal gene with the inductive interaction between the two components of the kidney rudiment? Reciprocal combinations of normal and "lethal" ureter with normal and "lethal" kidney mesenchyme were made in vitro; the existence of inductive and reacting potencies in both "lethal" components was demonstrated by the differentiation of kidney tubules in all combinations, although both the degree and the rate of differentiation were reduced in comparison with findings for normal tissue. With this experimental approach, therefore, it could be shown that the lethal gene had not suppressed the kidney inducing factor in the kidney rudiments nor their ability to react. The failure of kidney differentiation in the embryos homozygous for the mutation might be explained in a different way. It seems that in normal development the rates of differentiation of the two kidney rudiment components, ureter and kidney mesenchyme, are geared to each other in such a way that inductive interaction is made possible at the required time

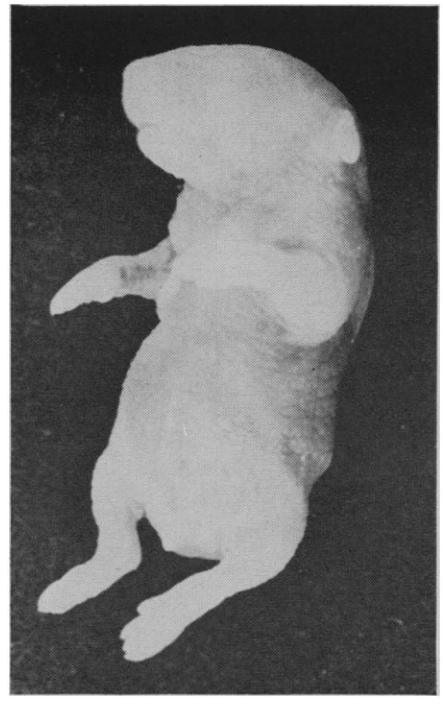


Fig. 1. A tailless and imperforate newborn mouse homozygous for gene *Sd* (× 3).

and stage. A change in these rates may interfere with this essential synchronization of inductive and reacting processes and result in failure of normal differentiation. Such a change of rates may have been caused by the mutation that suppressed kidney development.

Lethal Genes and Early Embryonic Differentiation

The study of lethal genes has made possible the further analysis of various causal mechanisms of early embryonic differentiation in higher organisms. Central among the problems that confront any student of developmental genetics is that of the mechanisms by which genes exert their effects on normal embryonic differentiation and inductive interaction between various parts of the developing embryo. An extensive series of mutations in the mouse, with lethal effects in early stages of embryogenesis, has furnished a wealth of material for such studies by virtue of the interference of the mutations with normal embryonic differentiation and normal inductive phenomena.

Much of our knowledge of the causal morphology of early mammalian development is based on analysis of the effects of this large series of lethal genes located in an abnormal region of chromosome 9 of the mouse. Genetically, these mutations are of considerable interest for various reasons. Their relatively high rate of mutation appears to be similar to the rate of crossing over in this abnormal *T* region (*12*). Consequently, it is assumed that rare crossing over changes the length or position of the abnormal region and results in alteration of the effects of a particular "mutated" *t* allele. Different lethal alleles result from different changes in the length of the abnormal chromosome region. Since lethal *t* alleles were first reported, a large number of new alleles have been discovered, all of them possibly the result of crossing over in the abnormal region of chromosome 9.

Individuals heterozygous for any two of these different lethal alleles are viable, and two such heterozygotes when mated with each other appear to breed true because of the operation of a balanced lethal system, in which both types of homozygotes are eliminated before birth by lethal gene effects and only the heterozygotes survive.

Balanced lethal systems have been demonstrated and described in *Drosophila* (*6*). Such systems lead to "enforced heterozygosis," since both homozygotes are inviable. In contrast to an ordinary population, where inbreeding results in a gradual separation of homozygous strains, inbreeding of individuals with balanced lethal genes continues to produce heterozygotes only.

Aside from the peculiar genetic features of this series of *t* alleles in the mouse, the main interest in these lethal genes with effects on the embryo derives from the fact that, because of their interference with specific processes of differentiation, they are tools for studying the relationship between chromosomal or gene structure and embryonic differentiation (*13*).

Figure 2 illustrates an embryo homozygous for one of the *t* mutations. A comparison with its normal littermate shows general retardation, overgrowth and abnormal differentiation of head folds, reduction of mesodermal trunk areas—for example, complete absence of somites—and overgrowth and duplication of the allantois. This homozygote does not survive beyond the stage pictured here. The differentiation

of neural tissue and structures, of somites, and of other mesodermal tissues is affected by this mutation; its analysis may illuminate many features of normal differentiation of the same elements.

The earliest lethal effects of any *t* allele were demonstrated in preimplantation stages where differentiation of morula to blastocyst was suppressed and where, on the cellular level, the concentration of RNA was decreased and the shape of the nucleolus was abnormal in cells of embryos homozygous for the allele.

Suppression of mesoderm formation and abnormal embryonic organization are characteristics of embryos homozygous for another lethal allele of this series. The correlation of abnormal notochord differentiation with abnormal neural tube development in embryos homozygous for the allele of this series that was studied first suggested the existence in mammals of an inductive relationship between notochord and neural ectoderm resembling that in lower vertebrates.

The problem of lethal gene action in this mutant was attacked experimentally (*14*) by explantation and in vitro culture of tissues from mouse embryos homozygous for the lethal gene, which ordinarily die around the age of 10 days of prenatal life. Such explanted tissues were capable of living and differentiating far beyond their potentialities for survival and differentiation in vivo.

Thus it was concluded that this mutation does not act as a "cell lethal," at least not in every cell of the homozygous embryo. The ability of tissues from this mutant to survive in vitro is in agreement with the demonstration (*15*) that the immediate cause of death of the homozygous embryo is the complete absence of umbilical and vascular connections with the mother and subsequent failure of processes of nutrition and waste-product removal. Consequently, it does not seem necessary to postulate an inherent defect of cells and tissues to account for the death of the embryo. It is impressive that in spite of the complete absence of umbilical connections with the mother, the homozygous embryo is able to develop up to the limb-bud stage—a finding which indicates the embryo's amazing independence of direct maternal blood supply during early stages of organ differentiation.

The appearance of duplications of various embryonic structures and rudiments in another homozygote of this series has been interpreted as a strong indication of the existence of organizer phenomena and inductive interrelationships during early processes of normal differentiation in mammals, similar to the phenomena and interrelationships demonstrated in lower vertebrates in the experimental analyses of Harrison and Spemann and members of their schools (*16*).

Both in vitro explantation and transplantation to various sites of a host may prolong the survival time of parts of embryos homozygous for a lethal gene. Thus, the differentiation potentials of cells and tissues beyond the stage at which the whole embryo ordinarily dies may be examined. In this way, effects of a gene other than those expressed in vivo are revealed. For example, the lethal gene "Splotch" in the mouse, which interferes with the differentiation of derivatives of the neural crest, causes death of the homozygous embryo at a stage before the differentiation of all derivatives of the neural crest is completed in the normal embryo. Transplantation experiments made it possible to study the differentiation potentials of neural crest derivatives, from mutants, beyond the lethal stage and to demonstrate, for example, abnormalities of melanophore differentiation; thus, melanophores were added to the list of neural crest derivatives affected by this mutation (*17*).

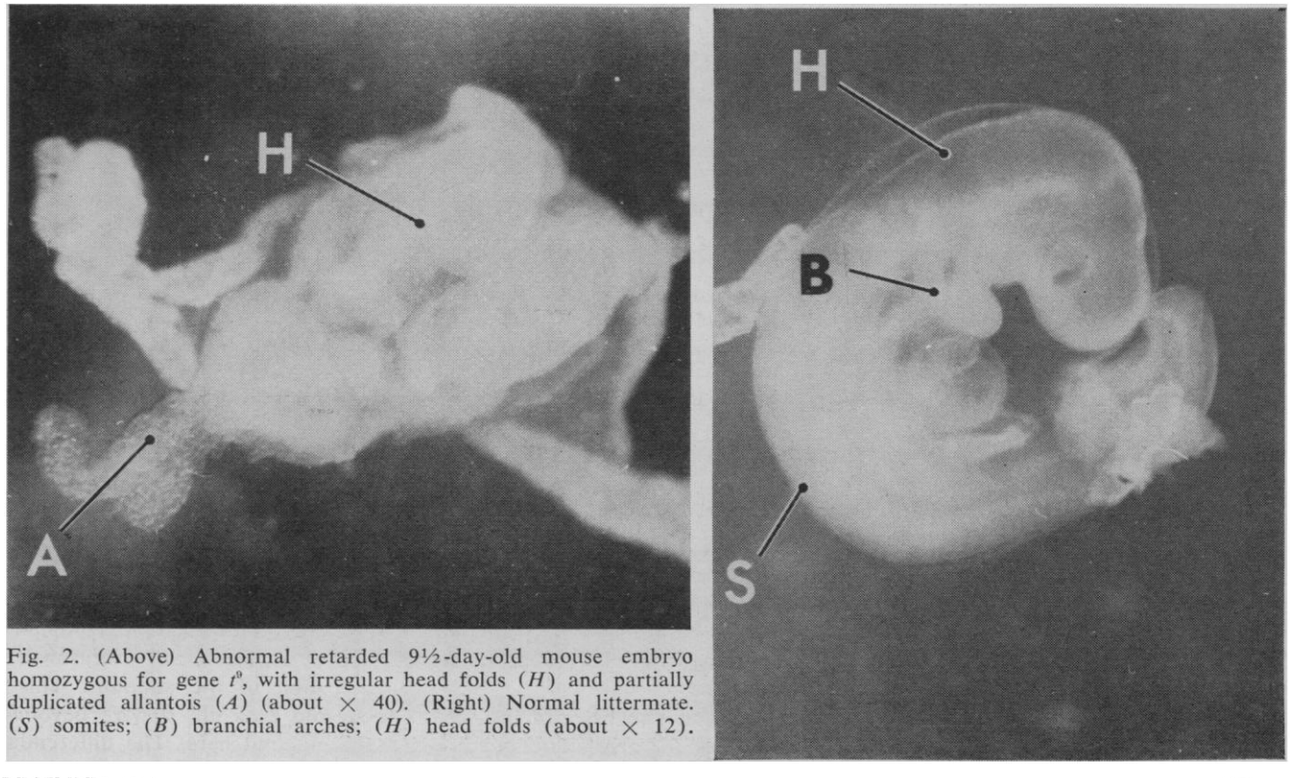


Fig. 2. (Above) Abnormal retarded 9½-day-old mouse embryo homozygous for gene t^0, with irregular head folds (*H*) and partially duplicated allantois (*A*) (about × 40). (Right) Normal littermate. (*S*) somites; (*B*) branchial arches; (*H*) head folds (about × 12).

Lethal Genes and Specific Tissue Differentiation

In a large number of lethal genes with effects on the embryo, these effects are expressed in a multiplicity of embryonic tissues, although general cell lethality can be excluded as the cause of death of the embryo. There exist various other lethal genes whose effects are concentrated on a particular tissue; among them are genes, for example, which interfere with erythropoiesis to an extent incompatible with survival. An interesting series of such lethal alleles is that which produces anemia of varying degrees in the mouse (*8*).

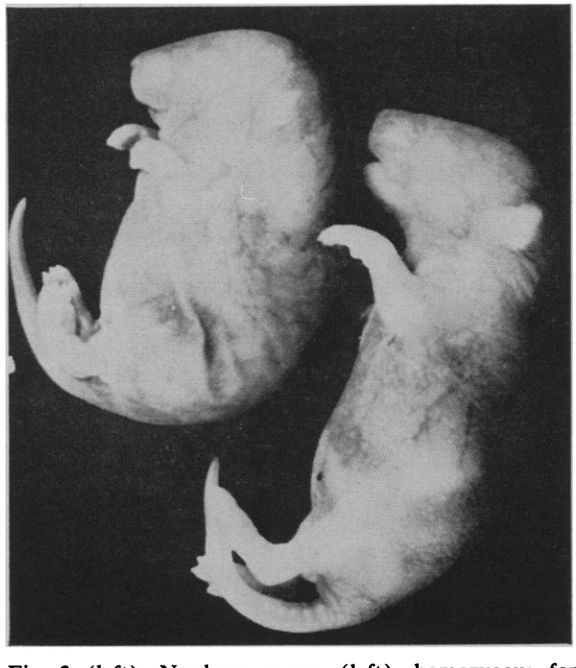
In man, the effect of a sex-linked conditional lethal gene seems to be restricted to the red blood cell, which in the mutant shows decreased activity of the enzyme glucose-6-phosphate dehydrogenase and is subject to premature breakdown and death. The conditional nature of this particular lethal gene appears from the fact that premature breakdown of the red cell occurs only in the presence of certain noxious agents, such as exist, for example, in the bean *Vicia fava* and in certain drugs (*18*).

The restriction of the effect of this "lethal" to the red blood cell may, of course, be simply an expression of the state of the reacting system. A deficiency of the enzyme glucose-6-phosphate dehydrogenase can have an effect only in those tissues where the enzyme is essential, such as the red blood cell.

A striking example of a lethal gene that affects one particular tissue is the mutation called "fetal muscular degeneration," (fmd) which is being studied in the genetics laboratory of the Albert Einstein College of Medicine (*19*). This recessive autosomal gene interferes, in the homozygous condition, with the early differentiation of the muscle cell to such an extent that normal skeletal muscle never develops. At birth, the lethal syndrome in the homozygote consists of various gross abnormalities of shape and structure (Fig. 3). Degeneration of the skeletal musculature, including the intercostal muscles, contributes to the inability to breathe and the subsequent asphyxia which is probably the immediate cause of perinatal death. The fetus is totally unable to respond to tactile or electrical stimuli, probably because of the muscular degeneration. In early developmental stages severe edema characterizes the abnormal fetus; the precise nature of the edema and its possible cause are as yet not known, but it may be correlated with the degeneration of differentiating muscle cells. Abnormalities of the myotubes in the earliest stages of muscle differentiation are actually the first observable deviations from normal. The correlation of abnormal differentiation of muscle with muscle degeneration and fetal edema makes this lethal syndrome of special interest for studies of differentiation of muscle, its biochemical basis, fetal muscle physiology and neurophysiology, and, possibly, the etiology of muscular dystrophies. It would be of great interest to apply methods and concepts developed recently in studies of the genesis of multiple forms of enzymes in the developing muscles of the chick embryo (*20*) in investigating the biochemical nature of the defect of muscle differentiation in mouse embryos homozygous for this lethal gene.

Cell Lethality and Autonomy of Lethal Effects

The concept of "cell lethal" mentioned in the discussion of explanation and transplantation experiments, usually refers to the expression of the lethal effect of a mutation on the cellular level in every cell. In the cases discussed, true cell lethality was thought to be excluded by the observation of survival and differentiation of explanted tissues beyond the survival and differentiation of the same tissues in vivo.



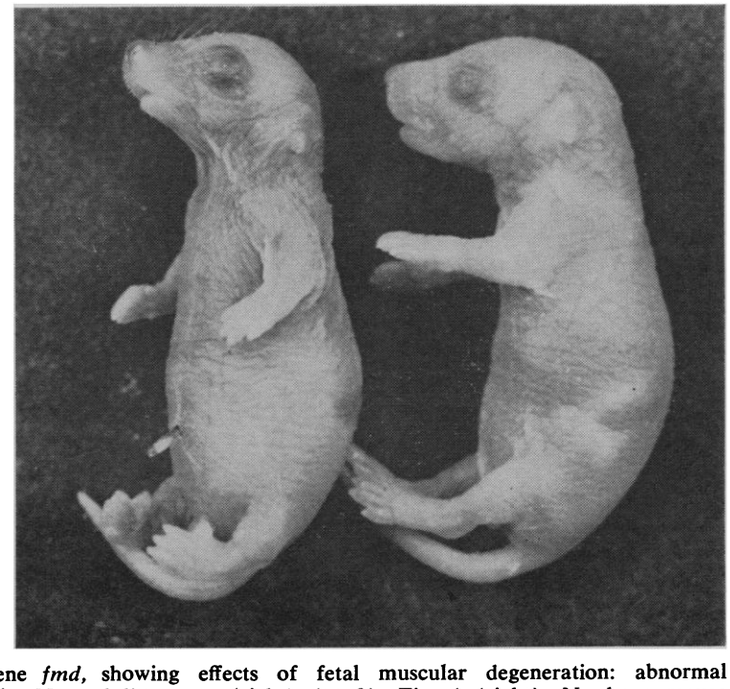


Fig. 3 (left). Newborn mouse (left), homozygous for gene *fmd*, showing effects of fetal muscular degeneration: abnormal shape, looseness of skin, typical limb position, microagnathia. Normal littermate (right) (× 3). Fig. 4 (right). Newborn mouse (left), homozygous for gene *pc* (the "phocomelia" gene), with phocomelic limbs and an abnormally shaped head. Normal littermate (right) (about ×3).

If transplantation of "lethal" tissues into a host of normal genotype, or explantation in vitro, fails to increase the survival time and the degree of differentiation, the lethal effect is referred to as "cell lethal" and considered to be autonomous. There seems to be a fallacy in an argument which correlates autonomous behavior of mutant cells—that is, the failure of correction of the defect by external factors—with lethal gene effect at the cellular level.

The autonomous or nonautonomous character of a lethal gene depends on many factors, such as the material nature of the genetic defect, or conditions extraneous to the gene effect, such as permeability, which permit or exclude correction of the deficiency through the supply of enzymes or substrates from outside the cell.

It is conceivable, for example, that a "cell lethal" with the phenotype of an essential-enzyme deficiency based on a structural gene mutation might show autonomous behavior and be incapable of being corrected under conditions in which substrate concentrations vary. The same variations of substrate concentration might, on the other hand, reveal nonautonomous behavior and correct for a defect in cells with the same lethal phenotype, caused, in this case, by mutation in a regulatory gene.

Both examples illustrate lethal gene effects on the cellular level—that is, effects of "cell lethals," one of them autonomous and unaffected by certain external conditions, the other nonautonomous and capable of being corrected by extraneous factors.

In unicellular organisms, differences of reaction of mutants of the same cistron in respect to suppressibility of phenotypic expression by external suppressors have been demonstrated in *Escherichia coli* (*21*). Certain alkaline phosphatase negative mutants in *E. coli* are suppressible—that is, they show nonautonomous behavior as the result of experimental manipulation; other mutants of the same cistron are not suppressible and are therefore autonomous. As Garen points out, different primary gene action in the case of these different mutations may account for the difference in their reactions.

When the problem is one of lethal gene action in higher organisms, the situation is obviously even more complex because of the interaction of various parts of the organism both during embryonic differentiation and in the mature state.

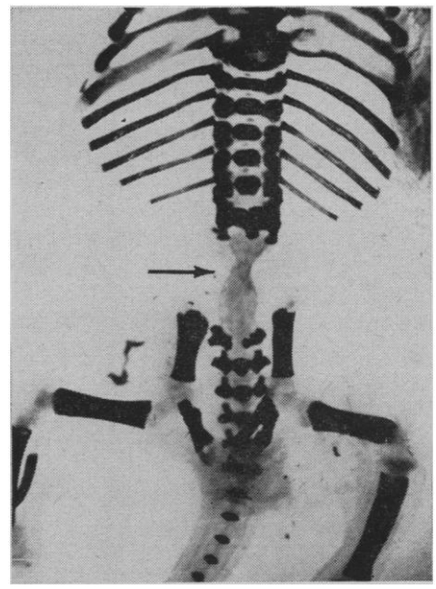


Fig. 5. Skeleton of newborn "lumbarless" mouse, stained with alizarin red. Several vertebrae of the lumbar region are missing, and the spinal cord shows a constriction, as indicated by the arrow. (about × 7).

It is important to realize that results of transplantation or explantation experiments which change the mutant cell's environment serve to determine autonomy or nonautonomy of the lethal gene on the phenotype level only and in no way reflect the nature of the gene itself. On the other hand, studies, such as those just mentioned, of the suppressibility of mutations within one cistron are concerned with problems of autonomy on the gene level and have helped in the analysis of the nature of the mutated gene itself, not just of the nature of its phenotypic effect.

Lethal Genes and Differentiation of Embryonic Patterns

In the thinking of embryologists interested in the causal analysis of development and differentiation, the phenomenon of aggregation of cells into definite patterns has played an important role. The possible role of genes in such pattern formation has been the object of many studies in the past. Recent methods of cell disaggregation and reaggregation have served to revive interest in the significance of developmental patterns; studies of the genetic basis of cell aggregation behavior appear to be promising. Much material that is valuable in this connection is provided by lethal mutations in which the main effect of the lethal genes appears to be a disturbance of the normal formation of aggregates of cells in early differentiation.

One such lethal gene is "phocomelia," which, in the homozygous condition, causes death of the newborn within a few hours after birth as the result of a cleft palate (*22*). Phocomelic newborn mice show severe shortening of the extremities and other skeletal abnormalities (Fig. 4), reminiscent, to some extent, of those described in human babies of mothers who had taken thalidomide. Studies of the development of the limb rudiments in embryos homozygous for the lethal gene revealed, as the first observable deviation from normal, an abnormal pattern of mesodermal cell aggregates in the early limb rudiment. Subsequent differentiation showed no specific abnormalities, such as cell degeneration or deficiencies demonstrable by histochemical methods; abnormal differentiation expresses itself primarily in disturbances of the pattern of development in time and space. This lethal gene seems to produce its effect by interfering with the normal pattern of mesodermal cell aggregation in the early embryo. It would be interesting to examine this hypothesis further with the help of methods of cell disaggregation and reaggregation.

Recently a new mutation in the mouse called "lumbarless" was discovered in this laboratory—a conditional lethal mutation which causes the death of certain homozygotes in early adulthood as the result of paralysis of the posterior part of the trunk and the posterior extremities. Morphological studies revealed abnormalities of the lumbar spine of various degrees of severity: lumbar vertebrae may be missing altogether, or they may be so abnormal that they appear jumbled. The spinal cord is severely abnormal in the lumbar region, and much thinner than in the normal animal (Fig. 5). The paralysis seems to be a consequence of the abnormality of the spinal cord. Our interest in this particular mutation lies in the implications it may have for studies of the inductive relationship between neural tube and cartilage-forming somite material. It has been demonstrated (*23*), with methods of organ tissue culture, that the spinal cord is able to induce, specifically, cartilage in somites; a nucleotide-containing component extracted from the spinal cord seemed to be responsible for promoting such cartilage formation.

The lethal mutation affecting lumbar differentiation of spine and spinal cord may serve as a tool in further studies of inductive interaction between spinal cord and somites in the embryo. Questions to be asked might include the following: Does an abnormality of the neural tube in the lumbar region precede abnormalities of differentiation of the somites in the same region? Or do the somites in the lumbar region of the mutant embryo react abnormally to a normal inductive stimulus of the ventral spinal cord? If the latter is the case, is the eventual abnormality of the spinal cord, as found in the newborn, caused in turn by the abnormal somites? In this way various problems in the analysis of the normal system of inductive interaction between spinal cord and somites may be approached with the help of this lethal mutation.

Phenocopies

Analysis of the mechanisms of effects of lethal genes has gained much from the use of so-called phenocopies. A phenocopy is an abnormality which, in a normal genotype, closely copies the appearance of a disturbance caused by an abnormal gene. It may arise sporadically, for unknown reasons, or it may be produced experimentally. Similarities between phenocopies and gene-controlled abnormalities open a way of approach to the question of pathways by which particular congenital malformations arise, whether they be gene-controlled or caused by external agents. With the use of phenocopies, an attempt was made to analyze the effects of a lethal mutation in the chick, the so-called Creeper mutation that results in severe skeletal abnormalities which can be "copied" experimentally by injecting insulin into the developing chick embryo (*24*). Although it is not possible as yet to ascribe the effects of the lethal Creeper gene to a specific disturbance of carbohydrate metabolism, the experimental production of phenocopies by the injection of insulin has served, first of all, to focus attention on carbohydrate metabolism as the possible site of the lethal effect; it has furthermore contributed to knowledge of the chemical embryology of carbohydrate metabolism and has called attention to the chick embryo as an excellent experimental object for biochemical studies of differentiation.

Congenital abnormalities such as those produced in human babies by thalidomide are phenocopies of similar malformations resulting from abnormal gene effects. Studies of the responsible mechanisms in both groups may possibly lead toward identification of the causality of the defect.

Conclusions

In the foregoing discussion emphasis has been placed on the analysis of lethal genes on the phenotype level. It has been argued that lethal genes are fundamentally not different from other mutant genes but that in their effects they are the most extreme representatives of a wide spectrum of mutations. Lethality, therefore, is not inherent in the lethal gene; it depends to varying degrees on other genes and the environment.

Because of their effects on development and differentiation, lethal genes offer particular promise as tools in the investigation of problems of cell differentiation and cell genetics. Study of lethal genes in higher organisms has aspects different from such study in microorganisms, primarily because of the phenomenon of interaction between cells and tissues which in embryonic differentiation provides one of the most significant causal mechanisms. Similarly, the existence of regulation, another causal mechanism of differentiation, gives special characteristics to the problem of lethal gene effects in multicellular organisms, both during embryogeny and in adult life.

The study of lethal genes in higher organisms is concerned with the analysis of gene expression beyond the molecular level of gene action. By providing aberrant processes—frequently a means whereby normal processes are illuminated—lethal genes have already proved themselves valuable tools in the analysis of causal mechanisms of development. In the future, studies of cell differentiation, in the focus of interest at present, will profit greatly from the use of genetically controlled aberrations of normal differentiation, and investigation of somatic cells that have been steered into abnormal channels of differentiation by lethal genes will no doubt contribute to progress of the analysis of the molecular basis of somatic cell differentiation, as well as to somatic cell genetics.

In recent years it has become obvious to all those actively engaged in studies of molecular biology that the problem of cell differentiation is still far from being solved, and that any general theory or model of gene action must be able to account for phenomena of cell differentiation. In this connection, lethal genes in multicellular organisms may well turn out to be valuable tools of analysis, providing experimental material in which abnormal cell differentiation occurs in combination with phenomena of interaction and regulation during embryogenesis (*25*).

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