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Autor: [s.n.]

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V. THE SIGNIFICANCE AND PREDICTIVE VALUE OF EXPERIMENTAL FINDINGS

A. General mechanisms of teratogenesis

A large variety of mechanisms can be implicated in the teratogenic action of environmental agents including chemicals. Theoretically, the possibility of a direct action on the embryo or foetus or an indirect action on the embryo or foetus via the modification of the maternal metabolism exists.

1. Direct action on the foetus: A direct action on the foetus is possible since many chemicals given to the pregnant animal reach the embryo without being modified; such transfer applies to drugs like certain antibiotics, sulfonamides and thalidomide.

2. Indirect action on the foetus: It is perhaps through an indirect action on the endocrine balance of the foetus that compounds like steroid hormones can induce virilization of human foetuses. Modification of the foeto-placental unit by biogenic amines can produce malformations by reducing placental transfer. A modification of the foetal nutrition can be suspected for certain chemicals, like trypan blue. In this case the particulate dye is retained in the visceral yolk-sac endoderm, which plays a very important role in nutrition of the embryo.

The possibility that inhibition of embryotrophic nutrition may result in the production of congenital malformations could also apply to other compounds that may act as inhibitors of constituent enzymes of the foetal membranes.

Drugs that modify maternal and possibly foetal metabolism, like hypoglycaemic or hypolipidaemic agents or compounds which influence lysosomal mechanisms (e.g. certain detergents), frequently cause congenital malformations. Consequently, it has been suggested that such anomalies are connected with failure, or lack of production, of a basic cell constituent.

Malformations may further be associated with production of an abnormal cell constituent, a protein or nucleotide. As has been suggested by CHAUBE and MURPHY (1968), antimetabolites may induce malformations by competitive action on nucleic acid metabolism. By this effect they may modify specific protein synthesis and act in a way comparable to that of the genetic factors.

Another factor involving maternal reactions may originate from metabolic interactions between various drugs, pesticides, food additives and a variety of environmental chemicals. These effects are due to activity of the microsomal enzymes, particularly of the liver, that may be stimulated or inhibited by chemicals that are metabolized on the cytoplasmic membranes. In this way, substances foreign to the body may modify the metabolism of other chemicals that are administered at the same time.

B. Mechanisms responsible for differences in susceptibility

Whatever the general mechanism of the noxious effect on the conceptus, the teratogenic action ultimately results in an impairment of normal processes of development, and the final response depends principally on the susceptibility of the animal, which is extremely variable: some species are highly resistant to one factor and sensitive to an other; different strains in the same species can be widely different in susceptibilities, and even in the same litter some embryos are quite normal, others are dead and others show malformations.

This can be explained at least partially on the basis of genetic differences and by the time factor involved in the particular type of interaction. This moment may be critical because of the strictly programmed cascade of intertissular reactions resulting in differentiation, and also because of the different susceptibility of various phases forming part of the growing cell populations.

C. Genetic susceptibility

The genetic aspect of susceptibility to teratogens has been partially resolved by a number of experiments using pure strains with known sensitivity to teratogens (Fig. 7).

1. Mendelian susceptibility: Hybridization experiments on inbred strains showed that susceptibility to a drug may depend on one or several genes the effects of which can be followed through successive generations. The following has been established:

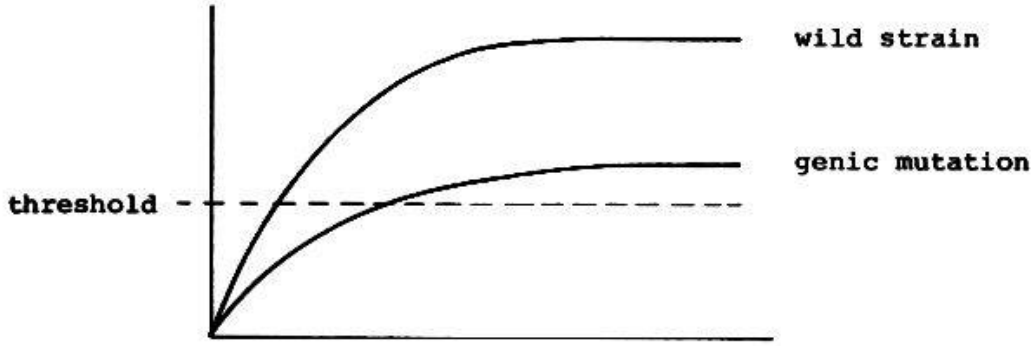
- The susceptibility is not a general one: the gene action is organ-specific. For instance, 6-amino nicotinamide induces a high percentage of vertebral anomalies in a mouse strain but practically no cleft palate.
- The tendency to malformation of one organ may depend on several genes, each being affected by various teratogens: a strain susceptible to one agent may prove to be resistant if a different teratogen is used. For instance, C 57 BL mice show 19% cleft palate when treated with cortisone, but none with galactoflavine, which induces 61% cleft palate in DBA mice.
- The expression of these genes may be more or less affected by the genetic background, as was demonstrated by successive cross and backcross between two inbred strains (which permits the "transplantation" of a mutated gene into another genome). In hybrid mice the genome inherited from the father or from the mother may play a different part.

These facts indicate that it is not possible to predict the susceptibility of one breed or strain to a compound from its known susceptibility to another compound however chemically related the two may be.

2. Polygenic heredity: This type of heredity is widely represented for a number of characters in man; it is certainly responsible for the susceptibility of a conceptus to different exogenous actions constituting perhaps the more important process in the multifactorial aetiology of congenital malforma-

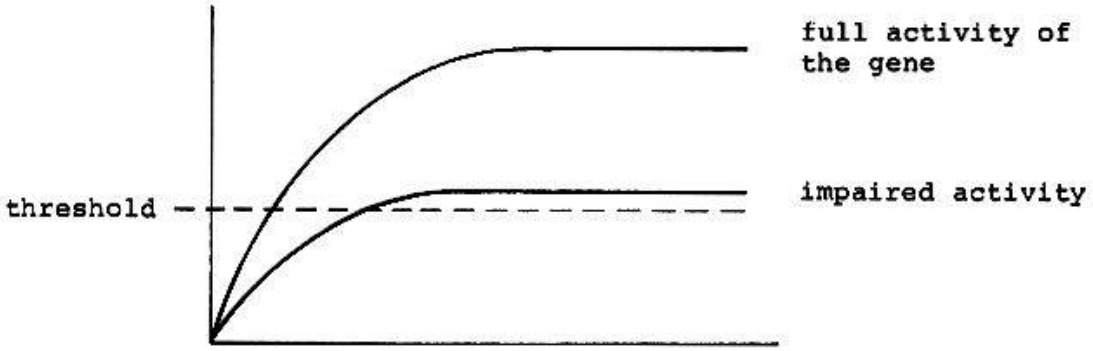
A = Genetic activity controlling the development of an organ

1. Mendelian susceptibility



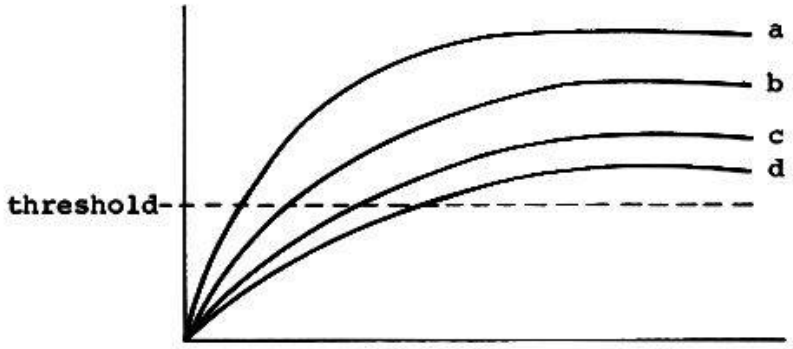
1- The new gene resulting from the mutation has a lower activity. It is close to the threshold. A "weak" teratogenic agent can bring it below the threshold, resulting in a malformation.

2. Action of the genetic background



2- The activity of the gene is impaired by an unfavourable genetic background. The susceptibility to teratogenic agents is increased.

3. Polygenic inheritance



3-A great number of genes with an additive action are implicated in the normal development of an organ. The dispersion of these genes in the population is a Gaussian one so that the genic activity is a continuous variable. The individuals having the lower number of genes at the extreme end of the variable (c,d) show the highest susceptibility to a teratogenic agent since the genic activity is close to the threshold.

Fig. 7. Models for the different genetic mechanisms of susceptibility to teratogenic factors.

tions. The results of extensive familial studies suggest that the usual type of malformation depends on a polygenic type of heredity. This shows a transmission which does not fit the Mendelian laws. In man, characters such as intelligence, eye colour, skin pigmentation and a number of pathological states are determined by a more or less considerable number of genetic loci having an additive action, the distribution of which in the population is a continuous Gaussian variable. Congenital malformations seem to depend on a continuous specific susceptibility, the small part of the population at one of the extreme portions of the Gaussian curve being the one that malformed. The elegant experiments of WALKER and FRASER (1956) on the influence of cortisone on cleft-palate induction have demonstrated the mechanism of such an action in teratogenic processes. The authors used two strains of mice: the A/Jax strain showing 15% of spontaneous cleft palate and strain C 57 BL showing none. By measuring the speed of the movement of the palatal shelves, palatal closure in the two strains was found to be normally distributed but the medium speed was significantly higher in C 57 BL mice. When treated with cortisone, the A/Jax strain produced 100% cleft palate and the C 57 BL only 17%. These results were explained on the basis of a threshold mechanism. The speed of the inward movement of the palatal shelves must be high enough to bring them together before the maxillary bodies have grown too large. When the speed is too low, the shelves do not meet at the right stage and cannot fuse. The threshold is defined by this critical interval. In C 57 BL mice all the animals are above the threshold: in A/Jax mice 15% are under and present spontaneous cleft palates. Cortisone induces a shift towards lower speeds so that 100% of A/Jax and 17% of C 57 BL are now below the threshold (Fig. 8).

This example affords some insight into the chief mechanism of the different incidence rates of cleft palate in different human races, and the tendency for cleft palate to occur more often in a family with a previously affected member with a risk which does not fit into the Mendelian laws.

Different mechanisms may be integrated in the model proposed in Fig. 6. Since teratogenic substances may act by interfering with the expression of genes commanding the development of an organ or system, a threshold effect would explain the occurrence or non-occurrence of a congenital malformation, admitting that the normal development is possible only if the genic activity is above the threshold. The closer this activity is to the threshold in normal conditions the greater will be the susceptibility to the teratogen.

This model is obviously an oversimplification but it may help to explain some of the phenomena underlying the extreme diversities in teratogenic response.

D. Chronological factors

1. Tissue interactions

In vitro studies with organotypic cultures of embryonic organs have disclosed very elegantly the complex sequences of tissular interactions which

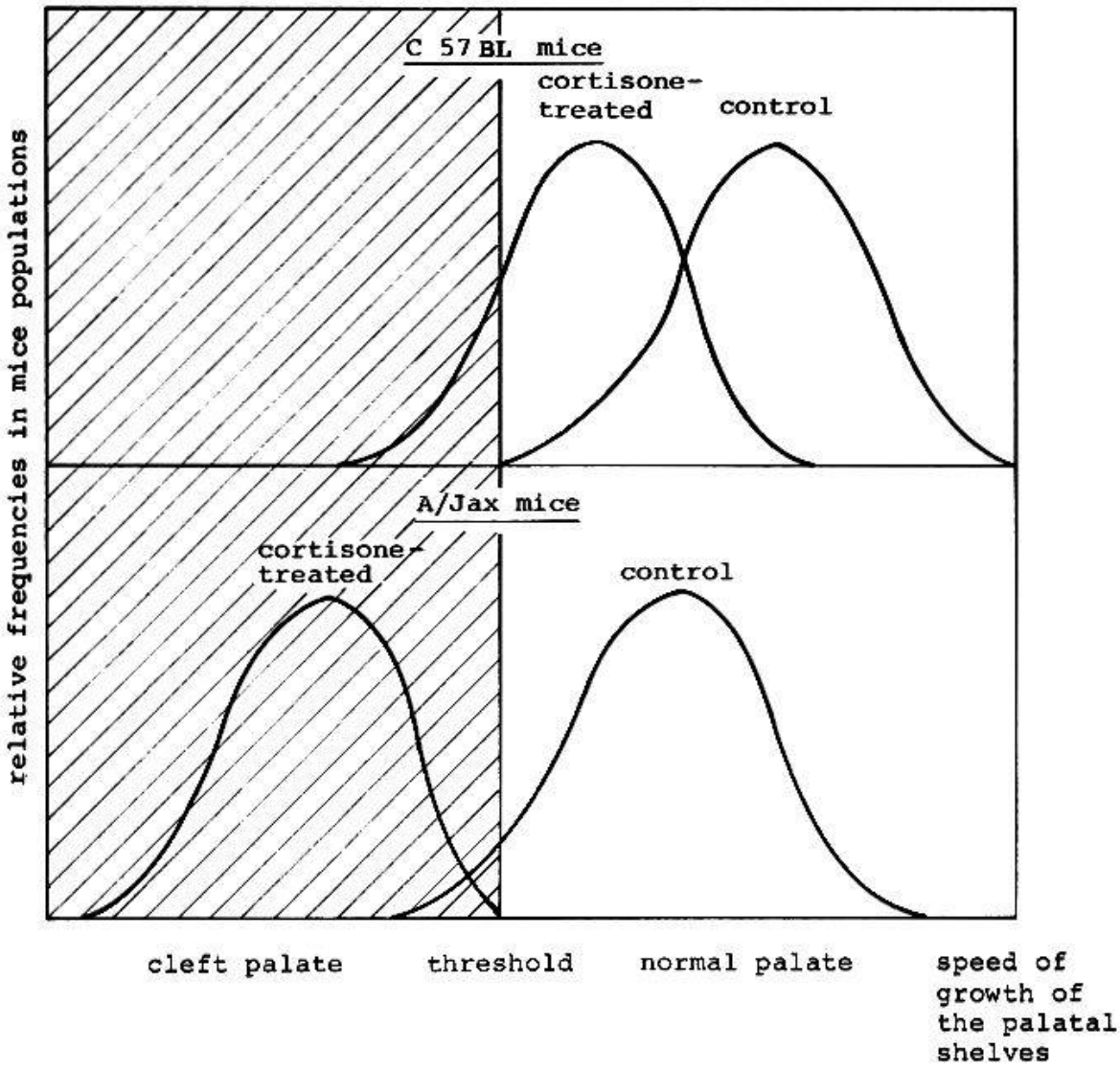


Fig. 8. The postulated mechanism of the differential susceptibility of two inbred strains of mice to cortisone concerning the induction of cleft palates.

Spontaneous induction of cleft palates in	C 57 BL = 0
	A/Jax = 15%
after cortisone:	C 57 BL = 17%
	A/Jax = 100%

underlie organ differentiation. These sequences are subject to a very critical timing. For instance, the differentiation of the different parts of a leg entails separate and successive inductive actions of the epithelium of the limb bud upon the mesenchyme. These different actions occur sequentially and each of them continues for a limited amount of time. If the mesenchymal reaction is blocked during the short time when the inductor for the proximal part of the limb is produced it will affect the distal part so that a phocomelia will appear. As these developmental changes are very rapid, a short difference in the moment of action of a teratogen may result in quite different responses. This factor may be one of the determining parameters which may explain the variable incidences of malformations in the descendants of

mothers of the same strain treated with the same dose of the same teratogen at the same stage of gestation: a small dissimilarity in the ovulation time may account for such differences.

2. Cell kinetics

Still more subtle chronological factors may be involved in the response of proliferation tissues. A number of teratogenic agents are known to interfere directly with cell multiplication. Recent progress in cell kinetics provided a new insight into the modalities of this action on the cell cycle (Fig. 9). A great amount of precise data was obtained through the study of tumour-cell multiplication and of the action of different drugs which impair this multiplication. These studies afford a model which can be used in embryology. Impairment of the multiplication of populations of cells in a developing organ results in a malformation.

These substances may be classified into: cycle-specific agents which act on any cell in its generation cycle, and phase-specific agents which act very specifically on one phase of the cell cycle. For instance a compound specific for the S-phase will act only on those cells which happen to be present in S-phase at the time the agent is present.

The teratogenic action depends again on a threshold effect determined by the number of cells entering the sensitive phase during the time the teratogen is present. If the agent intervenes at the beginning of a multiplication wave at one stage of organogenesis, it is easy to imagine how a very small difference in the beginning of the wave in embryos of the same litter may modify the cytostatic effect(s) of the antiproliferative agent. This offers wide possibilities of experimentation. Some interesting information is already available on the kinetics of embryonic cells. KOHLER (1970) showed that the speed of the generation cycle of embryonic cells in the rat varies considerably and rapidly in the course of embryonic life. Between day 11 and 12, the embryonic cells are engaged in an exponential growth, the duration of the whole cycle being 8 hours (3 cell generations in 24 hours). During this period of time the G1 phase is probably missing, most cells being in G2 and late S-phase. These modalities of the cell kinetics are changing from one day to the next. Such data are quite a valuable aid towards understanding how surprising differences may be shown by embryos of the same litter and therefore of similar genetic background that are exposed to the same environment through the same maternal organism.

E. Selection of compounds to be tested for teratogenicity and predictability of experimental data

1. Are there priorities in teratogenic drug testing?

As a general rule new drugs are tested in laboratory animals for possible effects on the embryo or foetus. Such tests should be a part of the routine preclinical testing if women of child-bearing age are included in the clinical trials.

Theoretically, any substance to which pregnant women might be exposed at concentrations high enough to cause a systemic effect should be properly evaluated by means of animal tests. In practice, because of the limitation of available testing facilities this may not be feasible.

Certain characteristics of a compound must be taken into account when discussing priorities, e.g. chemical structure and intended use. If, for instance, a new drug is chemically related to a substance or a group of substances known to have teratogenic properties, an initial comparative investigation is essential. Even if these tests indicate a lack of teratogenic potential, the negative results of extensive tests should be available before the conclusion is drawn that the compound is reasonably safe for use.

Intended clinical use in pregnant women is an important factor in the setting of priorities for teratogenicity testing. In this case the need for accuracy in estimating safety is particularly great. However, fixed rules of testing may be of little value in the individual case, since they can never cover all relevant factors; they may even produce a feeling of false security.

The question has been raised whether animal tests for teratogenicity could be omitted from the testing programme for new drugs intended to be used only by men, or by women not likely to become pregnant. This is generally not advisable, since apart from the fact that such drugs may unintentionally be administered to or taken by pregnant women, some information about the effect on the embryo and foetus may be desirable for the understanding of the biological profile of the compound.

Naturally occurring substances are generally considered to have rather low priority for safety evaluation in the animal. This may not be valid if "unphysiologically" high doses are used to produce a therapeutic effect and if therefore the pharmacokinetics are different from the "physiological" situation.

In the case of a drug having life-saving properties, teratogenic effects are considered to be only of scientific interest. Anti-neoplastic drugs are mentioned as examples.

A special group of drugs is those already long in use in human medicine. Very extensive clinical experience is available which carries more weight than the results of a comparatively small number of experiments in laboratory animals. Tests in animals of such drugs for safety evaluation are generally considered to have a low priority. From a scientific point of view, however, it is considered valuable to obtain information on teratogenicity studies in animals with well-known drugs. Such information may also be important for the assessment of the predictive relevance to humans of teratogenicity experiments in general.

2. Evaluation of teratogenic potential

Doubts have been cast on the relevance and predictive value of teratogenic testing. However, there is no alternative means of obtaining some guidance when evaluating a new drug or other compound (e.g. pesticides, food additives, etc.) to which man may be exposed.

One of the main difficulties in the extrapolation of experimental results to man lies in the different reactions of various animal species. These difficulties can be partially overcome by using several species and a large enough number of test animals to produce statistically valid data.

It has been suggested that animal species should be used whose pharmacokinetic behaviour and metabolism are comparable to that of man. Since this requirement cannot readily be fulfilled its practical meaning in present-day teratology is limited.

A vast amount of data is available to demonstrate that results obtained in rodents and lagomorphs may be considered meaningful in practical terms. The predictive value of experiments in these species seems to be at least as great as that of experiments performed in other mammals, including non-human primates. Apart from the fact that lagomorphs are sensitive to thalidomide, it should be brought to mind that the seeming similarity of reaction of primates and human beings to this drug is no phenomenon that can be generalized. It may be incidental, like the similarity between rodents and humans in their reaction to folic acid antagonists, for example. Taking into account the present uncertainties regarding primates as test animals it is felt that it is premature to recommend monkeys for routine teratogenic drug testing. It would be even more difficult to recommend other non-rodent species for tests, because there are insufficient data available on reproductive physiology and experimental teratology.

In the case of certain drugs, for example sex hormones, investigations in monkeys may be of scientific value, since in respect of biological activities rodents are not directly comparable to primates. It would be futile however to correlate the metabolism of the compound with biological effects since not enough information is available on any species, including humans, to allow these comparisons to be made. In evaluating the results of teratogenic tests due account should be taken of the pharmacodynamic and metabolic characteristics of the compound. In order to test the susceptibility of the particular type of animal used, the results should be compared to positive and negative controls.

The use of more than one species is usually necessary because many known teratogens produce a positive effect in one species only. When a potential drug produces a teratogenic effect in rabbits and rats or mice there is rarely any difficulty in interpretation. Real difficulty may emerge when a potential drug or other chemical substance produces a teratogenic effect in only one of the animal species tested. Possible relevance for man has to be assumed, unless it is possible to explain the specific nature of the developmental defect, and it can be confidently stated therefore that it has no bearing on the situation of the human.

According to present knowledge, there is no single drug assumed to be teratogenic in man that has not also produced malformations in rodents and/or rabbits.

An evaluation of the teratogenic risk should be made by taking into consideration all the circumstances under which the malformations occur: frequency of the anomalies, dose-response relationship, and number of species in which abnormal foetuses are found. A comparison with the effects of reference compounds may be of value.

The present testing methods appear satisfactory from the point of view of safety evaluation. However experimental animals having a high teratogenic susceptibility may yield false positive results. Therefore, if anomalies occur only in one species, special attempts should be made to test the possible specificity of the effect. Signs of positive teratogenicity in animals do not necessarily prevent a new drug from being used in humans, not even in pregnant women, but they are regarded as evidence of a potential risk until they are shown not to be relevant to the human situation.

If no teratogenic potential is revealed in animal tests performed according to accepted scientific standards, the compound is generally considered to be "safe for use in humans". This term implies relative safety, i.e. safety at an estimated or assumed dose level. In principle, the degree of safety is related to the amount of information available. In consequence, the question of priority of testing for teratogenicity is not only whether or not animal tests should be performed but also to what extent they should be carried out.

The problem of teratogenicity can hardly be subjected to the consideration of benefit against risk, since the aim of teratogenicity studies is in principle to reveal a special type of biological activity, the relevance of which is not easily established.