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Evaluation of Drugs and Other Chemical Agents for Teratogenicity

JOINT REPORT OF THE EXPERT COMMITTEE
ON TERATOGENICITY TESTING AND EVALUATION

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I. INTRODUCTION

Twelve years ago, an unexpected side effect of a drug on the conceptus was suddenly revealed. The dramatic circumstances of this discovery, and the limited scientific knowledge that was available at that time about developmental physiology and foetal pharmacology, led to a rather pessimistic appraisal of this type of adverse and irreversible action on the growing embryo. The possibility of a teratogenic hazard, particularly one caused by drugs, tended to be overemphasized, almost to the extent of jeopardizing any further therapeutic progress. It seemed that the only way to escape the potential danger of drugs for the growing embryo, was to deprive women for half of their lifetime of the benefit of efficient therapeutic agents.

To meet the most urgent needs, the World Health Organization convened a scientific group to devise appropriate methods for the testing of drugs for teratogenicity (1967). General principles were elaborated. These recommendations still constitute the framework of the experimental methods in teratogenicity testing as well as the point of reference of many basic research programmes.

However, many difficulties still exist in teratogenic drug screening and in the interpretation of experimental results.

The Swiss Academy of Medical Sciences was aware of the fact that, in recent years, a deeper insight into the nature of teratogenic effects has been gained and the reliability of the experimental methods used in their detection has been enhanced. In the hope of using this knowledge to augment the predictive force of teratological studies and formulate a realistic policy towards the evaluation of teratogenicity of drugs and other chemicals, an expert committee has been convened.

The aim of the group was to analyse the circumstances in which a teratogenic accident can occur and to define the most accurate experimental methods likely to disclose noxious agents.

It was understood that any recommendations emerging as a result of the work of the committee should remain flexible enough to be amended following further progress in reproductive physiology and foetal pharmacology.

Practical screening methods are based on the available scientific knowledge. Therefore it was felt that the recommendations of the group should be elaborated in the light of the basic principles of teratology.

A critical analysis was made of the various aspects of teratology, from the genetic, the epidemiological, the physiological and the pharmacological points of view.

This survey revealed the complexity of the aetiology of congenital malformations and hence the difficulties encountered in epidemiological investigation of demonstrating a causal relationship between an environmental factor and a birth defect.

Since such investigations require very large samples and long-term studies, the experimental methods, despite their limitations, probably constitute a more realistic and efficient approach to the detection of the teratogenic potential of environmental agents, particularly of drugs and other chemical agents.

It is often very difficult to make a clear distinction in the aetiology of many malformations between environmental and genetic causes, since some genetic dispositions display a greater susceptibility to the teratogenic agents of the environment than others.

The basic principles of testing for teratogenicity are similar to those underlying the detection of toxicity in general, except that the action of an injurious agent on the embryo is more complex than its action on the mature organism. In teratogenesis one is dealing with two biological systems – the pregnant female and the embryo – the specific reactions of which may be completely different. For instance, a drug innocuous to the female may be apt to kill the embryo or produce congenital malformations. The problem is further complicated by the fact that all types of disturbed development may occur through spontaneous mutation. An effect of treatment can therefore only be assumed if the incidence of the particular change is significantly higher than that observed in controls, or in a large population suitable for comparison. Furthermore, a dose-response relationship has to be established.

Embryolethality, embryotoxicity and foetotoxicity are changes that result from entirely non-specific interference with intra-uterine development and growth. Effects of this kind are not indicative of a teratogenic action; they may be induced by a large variety of chemical substances provided that sufficiently high doses are administered. Non-characteristic effects in the young are frequently observed at dose levels that cause symptoms and toxicity in the mother animal.

The significance of results obtained in such conditions, the high dose effects, the statistical evaluation of experimental data, and the particular problems raised by certain drugs have been discussed in the light of the experience gained in various research centres.

Although there are still many gaps in our knowledge of teratogenic mechanisms, and it might consequently seem hazardous to predict drug effects on man from data on laboratory animals, there is at the present time no satisfactory alternative.

From the present experience it might be assumed that there is no basic difference in susceptibility to a given teratogenic agent between man and the various mammalian species. Nevertheless if a teratogenic effect is observed as a result of treatment under the conditions of a particular experiment, it must be borne in mind that such effects are very often species-specific.

For this reason great caution must be exercised in extrapolating from animal experiments to man. However, it cannot be excluded, *a priori*, that a compound found to be teratogenic in laboratory animals may have similar effects in the human being. This fact is set forth in a report of the World

Health Organization (1972): "Modern animal toxicity studies even when supplemented by careful human pharmacology studies and clinical trials, still fail to detect certain delayed effects, novel types of toxicity effects that may be unpredictable owing to genetic variables, interactions between disease and drugs, and interactions among drugs themselves".

Despite the complexity of the problems involved in teratogenicity, the results obtained with the teratogenic screening methods, which were devised only a few years ago, compare favourably with those of general toxicology.

Although the ideal animal species whose embryo would react in all circumstances like the human embryo is non-existent, there are good reasons to think that the present difficulties in the evaluation of experimental data will be overcome by a better knowledge of teratogenic mechanisms and future progress in foetal pharmacology.

II. GENERAL CONSIDERATIONS

A. Fundamental aspects of human epidemiology

Aetiologies of congenital malformations

The development of the embryo is the resultant of two factors: the genetic information, which contains the programmation of the whole phenotype of the future child, and the environment, which supplies the nutrients necessary for growth and differentiation of the embryo.

Hence, congenital malformations may be determined in four ways (Fig. 1).

– *Genetic malformations:* a defect in a genetic factor is produced at the origin by a mutation and transmitted hereditarily. The defective gene is expressed immediately in the phenotype if it is dominant, but remains hidden until a homozygous conditions comes about if it is recessive.

– *The chromosomal aberrations:* represent a gross imbalance in a genome and result in important and complex malformations though the individual genes are normal. For instance: trisomy 21 in which the presence of three 21-chromosomes instead of two induces the Down syndrome.

– *Exogenous malformation:* The genome is normal and well balanced but its expression is impaired by exogenous factors present in the environment and acting in the course of the embryonic development and having a teratogenous effect.

– *Multifactorial malformations:* No clear-cut genetic defect exists but the expression of one gene or several genes is modified by small doses of a teratogen which are harmless for the progeny of the most part of the population. In this case, the expression of the gene concerned is at the limit of the normal and a slight impairment caused by exogenous actions brings it below a threshold separating the normal phenotypic trait from the malformation. This instability in the expression of some genes is at the origin of most of the usual congenital malformations.

In man, recent investigations have shown that the proportion of pregnancies ending in foetal or neonatal deaths is approximately 25%. Among the living newborns the incidence of malformations has been estimated to be 3% to 5%. One fifth of these (1%) are assumed to originate from a genetic defect. The chromosomal aberrations represent about 0.5% and the great majority is represented by the exogenous and multifactorial malformations.

B. Genetic malformations

The nucleus of the fertilized egg contains two sets of genetic information: the paternal one and the maternal one. All this information is transmitted to every cell in the embryo. The first step in the differentiation of an organ consists in a selective "derepression" of a particular series of genes which characterize the specific activity of the differentiated cell. This selective

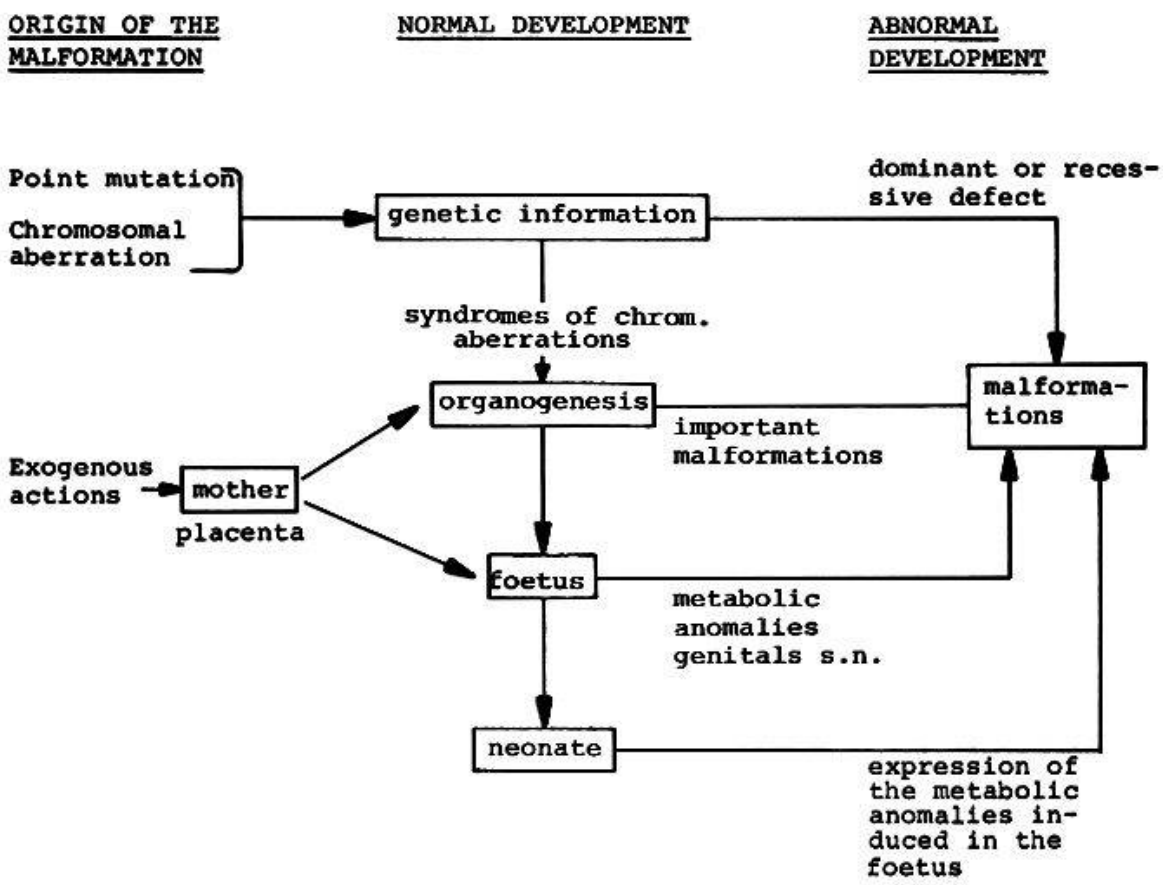

Fig. 1. Aetiology of the congenital malformations.

expression is controlled by the micro-environment represented by the cells of the neighbouring tissues. If a mutation exists in the active genes of the differentiating cell or of the controlling tissues, the normal development of the organ is impaired and a malformation may occur.

1. Spontaneous mutations

A number of agents are known to produce mutations, but many mutations appear without any recognizable cause. They are errors inherent in the replication process of the DNA molecules in which the genetic information is coded. The genetic defects described in man so far, both dominant and recessive, have been reported by McKusick (1971).

A mutation producing a dominant defect is immediately recognized: when a child bearing this defect is born of normal parents, it is concluded that the defect must have arisen through a genic mutation. In fact, the expression of a dominant gene may be more or less inhibited by other genes having a modifying effect on it. This influence of the genetic background explains the phenomenon of the "skipped" generation, in which an apparently normal child has received from his father a dominant noxious gene, which is not expressed in him (low penetrance of the gene), but may express itself fully in his son.

A mutation producing a recessive defect cannot be recognized as soon as it occurs: the heterozygous child bearing a recessive mutant gene is phenotypically normal; the new gene is transmitted and remains dormant in the population as long as a homozygous condition does not come about.

It is possible to calculate the general frequency of these affected genes in the general population from the incidence of affected individuals (HARDY-WEINBERG law)[1]. Calculations, made by very indirect approaches, indicate that each human individual bears in his genome three to eight recessive unfavourable genes. VOGEL (1970) arrived at an estimate of about 17.5 mutations/germ cell generation. This would mean that every individual carried an average of about 35 mutations which had originated in the germ cells of his parents. This "genetic load" could become still heavier in the future by the progress of therapeutics enabling abnormal individuals to survive and to reproduce, i.e. individuals who otherwise would have been eliminated through "natural selection".

An equilibrium is established between the mutation rate and the simultaneous loss of these mutated genes by poor fertility or death of the affected subjects. But this equilibrium is shifted when the heterozygous conditions confer a selective advantage or an increased fertility by comparison with the normal homozygotes. For instance, individuals who are heterozygous for sickle-cell anaemia have increased resistance to malaria compared to the rest of the population, which confers on them a selective advantage. While the proportion of sickle-cell anaemia genes in the population of countries with endemic malaria is abnormally high, it decreases slowly in the course of the following generations, when malaria is eradicated. This well-known example of heterozygous fitness is not unique and such a "balanced polymorphic system" may exist for a number of recessive conditions like phenylketonuria, mucoviscidosis and Tay-Sachs disease.

The mutations should theoretically occur randomly at any locus, but, in fact, the mutation rates differ for different loci and must be determined for each of them.

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[1] *The Hardy-Weinberg law:* Let us consider a pair of allelic genes in a population, where the frequency of the one gene is p and that of the other is q, the total sum being therefore $p+q=1$ (= 100%). If there is random mating (panmixis) in the population, the genotypes arising from the various gametic combinations will be represented in the next generation in the following frequencies:

$(p+q)^2=p^2+2pq+q^2$

where p^2 is the frequency of the normal individuals ("normal homozygotes"),

$2pq$ that of the heterozygotes ("carriers"), and q^2 that of the "affected homozygotes".

The relative proportions $p^2:2pq:q^2$ will remain the same also in the following generations, provided that there are no changes in the structure of the population.

Based on the proportion of the various genotypes, it is possible to calculate each of them, if we know the frequency of the manifestation (q^2) in the population.

2. *Determination of the mutation rate*

The mutation rate is *the number of modified genes per locus per generation.* It is often expressed as the number of modified genes per given number of gametes at each generation.

Two methods are used for its determination:

The *direct method,* which can only be used for dominant affections with complete penetrance. It consists in counting the number of affected children born of normal parents (n = "sporadic cases") and putting it in relation to that of the total number of births (N). The algebraic formula for the mutation rate (μ) is:

$$\mu = \frac{n}{2N}$$

The double number of born children (2N) in this formula is due to the fact that the mutation rate is not based on the number of individuals, but on that of the genes of the concerned locus.

Errors may arise from fluctuations in the penetrance of such genes and the existence of phenocopies induced by exogenous agents which are taken for mutations.

The *indirect method* is based on the assumption of equilibrium between new mutations and gene elimination by natural selection. It takes into account the frequency of the affected subjects in the population and their relative fertility. Thus, the quantitative determination of the gene loss by reduced fertility in every generation permits at the same time the evaluation of the mutation rate.

The value of both the direct and indirect methods depends to a large extent on the possibility of investigating a sufficiently large and representative population in order to ascertain all the bearers of a specific genetic trait. Other sources of error inherent in both methods may be heterogeneity of phenotypically similar affections as well as fluctuations in penetrance and expressivity.

In particular, as far as the indirect method is concerned, the postulated genetic equilibrium between new mutations and negative selection seems to be specially *questionable for autosomal recessive conditions.* Indeed, the elimination of disadvantageous genes may be partially or wholly offset by the selective advantage of the heterozygote with regard to certain diseases, so that the calculated mutation rate is higher than the real one.

The formulae for calculating the mutation rate are the following according to the different modes of inheritance (see Table 1).

Mutation rate in relation to paternal age

A number of mutations seem to occur preferentially in male germ cells and the age of the father has been demonstrated to be a decisive factor.

Table 1

Determination of the mutation rate according to the indirect method

autosomal dominant inheritance	$\mu = \frac{1}{2}(1-f)\,x$
autosomal recessive inheritance	$\mu = (1-f)\,x$
sex-linked recessive inheritance	$\mu = \frac{1}{3}(1-f)\,x'$
sex-linked dominant inheritance	$\mu = \frac{2}{3}(1-f)\,x$

$$\mu = \text{mutation rate} = \frac{\text{number of new mutants}}{\text{total number of alleles of a given locus in a population}}$$

f = relative fertility of the affected individual, when the average in the population f = 1

$$x = \frac{\text{total number of affected individuals}}{\text{total population (males + females)}}$$

$$x' = \frac{\text{number of affected males}}{\text{total male population}}$$

Regarding the incidence of achondroplasia, for instance, the age of the father was equal to or greater than 35. Similar correlations seem to exist also for acrocephalosyndactyly Apert and myositis ossificans.

Geographical "isolates"

The multiplication and expression of noxious genes are favoured by consanguineous unions occurring in geographical "isolates" where exceptional hereditary defects may be observed with an unusually high frequency and in a wide range of phenotypical variability. The "opening" of these isolates leads to a dilution of these gene-pools and lowers considerably the incidence of the recessive manifestations.

The following table (Table 2) lists the mutation rates in man according to VOGEL (1970). (The frequencies were taken only from investigations where the size of the material and the methodology of examinations were judged satisfactory). It appears from this table that all mutation rates may range from 10^{-4} to 10^{-6} fertilized gametes per generation. Therefore it must be assumed that these figures represent the general order of magnitude of the mutation rates in man. It is interesting to note that these estimates closely correspond to the figures for spontaneous mutations in mice reported by RUSSELL and SAYLORS (1961) (7.5×10^{-6}) and by SCHLAGER and DICKIE (1966) (2.7×10^{-6}).

Table 2

Selected mutation rates for human genes (F. VOGEL, 1964, 1970)

No	Affection	Population examined	Mutation	Number of mutants/ 1 million gametes
a)	Dominant mutations: More than one estimate			
1	Achondroplasia	Denmark	$1x10^{-5}$	10
		Northern Ireland	$1.3x10^{-5}$	13
2	Aniridia	Denmark	$2.9(-5)x10^{-6}$	2.9(-5)
		Michigan (USA)	$2.6x10^{-6}$	2.6
3	Dystrophia myotonica	Northern Ireland	$8x10^{-6}$	8
		Switzerland	$1.6x10^{-5}$	16
4	Retinoblastoma	England	$6-7x10^{-6}$	6-7
		Michigan (USA), Switzerland, Germany, Japan	$8x10^{-6}$	8
	One estimate only			
5	Neurofibromatosis	Michigan (USA)	$1x10^{-4}$	100
6	Polyposis intestini	Michigan (USA)	$1.3x10^{-5}$	10-30
7	Marfan's syndrome	Northern Ireland	$4.2-5.8x10^{-6}$	4.2-5.8
8	Polycystic disease of the kidney	Denmark	$6.5-12x10^{-5}$	65-120
9	Acrocephalo-syndactyly	England	$3x10^{-6}$	3
10	Osteogenesis imperfecta	Sweden	$0.7-1.3x10^{-5}$	7-13
11	Diaphyseal aclasis (multiple exostosis)	Germany (Reg.-Bez. Münster)	$6.3-9.1x10^{-6}$	6.3-9.1
b)	Sex-linked recessive mutations:			
12	Haemophilia	Denmark	$3.2x10^{-5}$	52
		Switzerland	$2.2x10^{-5}$	22
	Haemophilia A	Germany	$5.7x10^{-5}$	57
	Haemophilia B	Hamburg	$3x10^{-6}$	3
	Haemophilia A	Finland	$3.2x10^{-5}$	32
	Haemophilia B		$2x10^{-6}$	2
13	Duchenne type muscular dystrophy	Utah (USA)	$9.5x10^{-5}$	95
		Northern Ireland	$6.0x10^{-5}$	60
		England	$4.3x10^{-5}$	43
		Germany (Südbaden)	$4.8x10^{-5}$	48
		Wisconsin (USA)	$9.2x10^{-5}$	92
		Leeds (England)	$5.1x10^{-5}$	51

C. Chromosomal aberrations

They are true mutations. Classification in the truly genetic sense is as follows:

Aberrations of the genome: Polyploidy
Aneuploidy Monosomy
Trisomy
Polysomy

Aberrations of the chromosome: Aneusomy

i.e. Deletion	Inversion
Deficiency	Isochromosome
Dicentric chromosome	Ring chromosome
Duplication	Transposition
Insertion	Translocation centric fusion reciprocal

The following classification is used in clinical genetics:

- Autosomal aberration: Numerical or/and structural
- Gonosomal aberrations: Numerical or/and structural
- Mosaicism

Gene mutations are distinct errors of the molecular structure of the genes in the Ångstroem range, whereas chromosomal aberrations concern the morphological structure of the chromatids in the micrometer range, which can be diagnosed by special laboratory techniques under the light microscope. Special techniques of chromosome preparation are used to differentiate circumscribed parts in each chromosome.

Chromosomal aberrations originate *before* conception in the course of meiosis and spermatogenesis, *during* conception in the second stage of meiosis in oögenesis, or *after* conception in mitosis of early cleavage stages leading to mosaicism.

The estimates of frequencies and their importance regarding the aetiology of congenital malformations originate from two main sources:

- Cytogenetic investigations of spontaneous abortions
- Cytogenetic studies in series of newborns

According to JACOBS et al. (1972) at least 6% of all recognized conceptions seem to have an abnormal chromosomal constitution, but only approx. 0.5% may come to birth (Fig. 2, Table 3). BOUÉ et al. (1973) observed 921 (= 61.4%) chromosomal anomalies in 1,500 abortuses of the first trimenon. Similar percentages were obtained in a current prospective study of the Deutsche Forschungsgemeinschaft (DFG) concerned with "Pregnancy course and child development":

Up to April 1973 in a total of 13,948 pregnancies 1,341 (9.6%) spontaneous abortions were documented. Out of these, 180 spontaneous abortions could be analysed cytogenetically, i.e. 182 karyotypes resulted because of twinning in 2 cases. Karyotypes of the twin abortions were normal; for statistical reasons they were counted as two normal ones. 128 spontaneous abortions showed normal karyotypes, 52 had chromosomal aberrations (28.8%). The sample was subdivided into 70 early abortions up to the 13th week of pregnancy and 110 late abortions. The rate of chromosomal aberrations was

in early abortions	33 = 47.1%
in late abortions	19 = 17.3%.

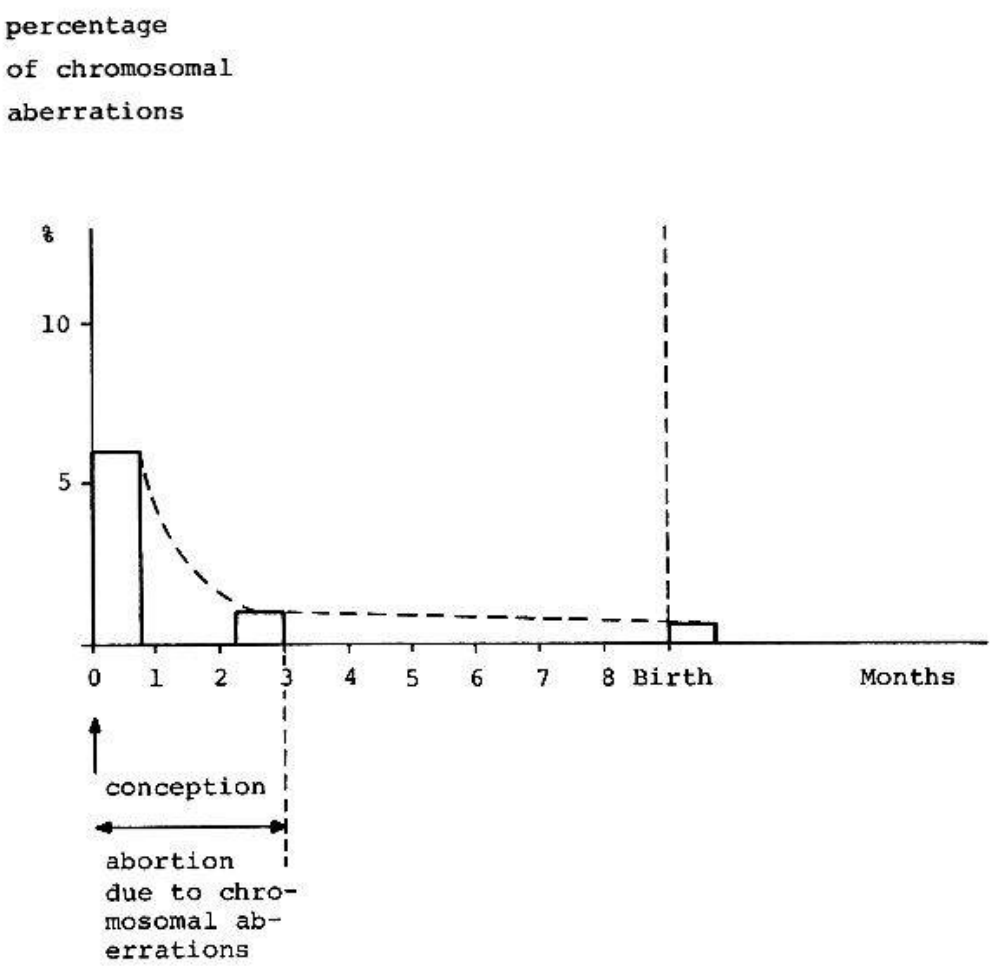


Fig. 2. The incidence of chromosomal aberrations in prenatal and postnatal life.

Table 3

Incidence of chromosomal anomalies in newborn surveys (consecutive hospital births) according to P. A. JACOBS et al. (1972)

	Numerical aberrations	Structural aberrations	Total live births
Autosomal	trisomies (21, 18, 13 and others) 0.13%	0.18% balanced translocations = 5/1 non-balanced	0.31%
Gonosomal	XO, XYY, XXY, XXX etc. 0.2%	0.008%	0.21%
Overall			0.52%

The distribution of types of chromosomal anomaly in comparison with the observations of BOUÉ et al. (1973) is as follows:

	Prospective study DFG		Boué et al.	
	n	%	n	%
Trisomies	27	51.9	495	53.7
Monosomies	11	21.2	141	15.3
Mosaics	7	13.5[1]	10	1.08
Polyploidies	5	9.6	240	26.04
Structural Aberr.	2	3.8	35	3.8
Total	52	100.0	921	100.0

[1] Incl. possible pseudo-mosaics because of growth of maternal normal cell lines.

The seeming discrepancy between the results of these series needs further clarification. In cases where the embryo was present development usually stopped around the 4th to 6th week p.conc. About 70% of those embryos which stopped developing before the 4th week p.conc. were malformed. Similar malformations are common to embryos with different karyotypes.

All autosomal and gonosomal numerical aberrations, but only 25% of structural anomalies are new mutations. From the data available so far, the rate of chromosomal aberrations due to new mutations has been calculated at 1.85×10^{-3} per gamete/generation. This may be underestimated.

Phenogenesis in specific chromosomal aberrations still remains largely obscure. Characteristic malformation syndromes can be related to specific numerical or structural anomalies, but single anatomical deviations from the normal range of variation seem to be quite non-specific. It is obvious that the normal command of the genome over embryonic development is disturbed by the lack or the addition of functionally important chromosomal material. This concerns whole chromosomes as well as parts of chromatids. Vogel (1973) pointed out the importance of bands and interbands of chromosomes as functional units of gene action. He formulated the hypothesis that "the main clinical symptoms of the syndromes caused by numerical or structural chromosome aberrations in man are not caused primarily by aberrant numbers of classic structural genes, but by disturbances in the function of DNA with control function".

1. Autosomal aberrations

According to Jacobs et al. (1972) the incidence of autosomal chromosomal anomalies is as follows:

Numerical 0.13% Structural 0.18% Total livebirth 0.31%.

Only a few types of autosomal numerical aberration may be compatible with intra-uterine development and live birth. Up to now they predominantly concern 3 trisomies, of which only one, i.e. trisomy 21, may be compatible with further development after birth. Trisomy 21, characterized by the

phenotype of Down's syndrome, is of considerable practical importance because the viability of the patients can be improved by care. This syndrome accounts for nearly one third of all severely subnormal children. The incidence in the DFG prospective study was 15:9,770 total births = 0.15%.

A cytogenetic classification of trisomy 21 can be made as follows:

- The regular trisomy 21 (3 free chromosomes 21):
  approximately 93% of all cases
- Mosaics normal cell line/trisomy 21 cell line:
  approximately 2% of all cases
- Centric fusion trisomy 21 with a D/G (predom. 14/21) or a G/G (21/22 or 21/21) translocation chromosomes:
  approximately 5% of all cases
- Partial trisomy 21:
  extremely rare

The condition of trisomy G in relation to clinical symptoms similar to the Down syndrome has recently been observed in the non-human primate, a female chimpanzee (Pan troglodytes).

Trisomy 13 (the Patau syndrome) and trisomy 18 (the Edwards syndrome) are semilethal; the aberrations are correlated with characteristic malformation syndromes.

Structural autosomal aberrations may be of clinical and social importance as they concern characteristic malformation syndromes that may be compatible with life and that are associated with mental imbecility.

Specific deletions in relation to well-defined syndromes are:

	Incidences
5p– (the "cri du chat" syndrome Lejeune)	1:50,000
4p– (the Hirschhorn-Wolf-syndrome)	1:500,000

18p– or 18q– (syndromes de Grouchy I or II) Observations in single cases.

The Philadelphia chromosome (Ph 1) correlated with acute myeloblastic leukaemia is in fact a chromosome 22 with a deletion in the distal parts of the long arms. The partial trisomies 8p or 8q, 7q, 9p, 22q– are observations in single cases. They, too, seem to be recognizable as specific malformation syndromes.

Somatic structural mutations concern chromosome or chromatid breaks and chromosome reunions, closely related to six inherited diseases: Fanconi's anaemia, Bloom's syndrome, ataxiatelangiectasia, glutathione reductase deficiency, Kostmann's agranulocytosis and pernicious anaemia. The cytogenetic data are by no means uniform; in each disease, the incidence of leukaemia is increased. According to SCHROEDER (1973) a change in the genetic material itself may create primary, yet unknown conditions for malignant growth.

2. Gonosomal aberrations

According to JACOBS et al. (1972), the incidences are as follows: numerical 0.2%, structural 0.008%, total livebirth 0.21%. Karyotypes 47,XXY and

47,XYY are predominant (approx. 1:900 of male births for each aberration), but they cannot be reliably recognized clinically before puberty. The relationship between X polysomies and specific syndromes depends mainly on the inactivity of the supernumerary X chromosomes. M. LYON's hypothesis of X inactivation in the female around the 16th day p.conc. nowadays may be understood as an incomplete inactivation of one or both X chromosomes mainly in the short arms, where the genes regulating whole-body growth are located.

The 45,X chromosomal constitution leads to the characteristic Turner's syndrome and may be recognized at birth. According to JACOBS et al. (1972) only one case in 9,456 female newborns has been found, but the incidence in spontaneous abortions is about 40 times as high.

Nine cases of triple X females in 9,456 female newborns have been observed by JACOBS et al. (1972); they are increasingly liable to psychosis in later life, otherwise they seem to be normal with their fertility intact. They may give birth to a child with the 47,XXY constitution.

In all the gonosomal numerical aberrations, mosaic mutations, with two or more cell lines either in combination with a normal cell line or with different aberrant cell lines only, could be observed. They are related to a high degree of variability in clinical manifestations. Structural gonosomal aberrations are very rare. They are only important as far as they concern the localization of specific functional gene sites; i.e. the localization of sex-determining factors on the Y chromosome.

SIEBERS et al. (1973) concluded from their comparative analysis of all cases so far observed that the genes for the initiation of testicular development are located on the proximal part of the long arm of the Y chromosome, while the genes responsible for the maturation of the testis are localized on the short arm of the Y chromosome.

3. Factors favouring the occurrence of chromosomal aberrations

Meiosis in the female already starts before birth; homologous chromosomes join in meiotic prophase and rest in dictyotene, for 2 or 3 decades, until the germ cell is stimulated to finish meiosis. During the long time of rest the pairs of chromosomes might be influenced by a variety of exogenous agents, i.e. X-irradiation, cytostatic or antimitotic chemicals and viruses. From this point of view, the increased rate of meiotic non-disjunction leading especially to trisomy 21 in the offspring of late pregnancies is readily understandable. In the male germ cells, meiosis starts at puberty and then continues. It has been shown in the male also that non-disjunction may occur in meiosis and may result in a conceptus with a regular trisomy 21.

Mitotic postconceptional non-disjunction may occur spontaneously or may be influenced by exogenous agents. This has been shown in experimental investigations with hamsters and mice (YAMAMOTO and INGALLS, 1972; ROEHRBORN and HANSMANN, 1973).

4. The value of chromosome studies

a) Possibility of an autosomal chromosomal aberration

- Differentiation of a chromosomopathy syndrome from monogenic multifactorial or exogenous causes
- Verification of trisomy 21
- The cause of multiple spontaneous abortions may be a reciprocal translocation in one of the parents
- Detection of the Ph 1 (22q–) in myeloid leukaemia
- Verification of a preleukaemic inherited disease by the detection of chromatid or chromosome breaks and rearrangements or pulverization.

b) Possibility of a gonosomal chromosomal aberration in certain endocrine disorders

- Intersexual external genitalia in the newborn, all types of intersexuality in children and adults (exclusion of adrenogenital syndrome)
- Inguinal hernia in females (exclusion of testicular feminization)
- Retarded puberty in females of small stature
- Primary amenorrhoea
- Retarded puberty in males with small testes and mental retardation
- Small testes and/or sterility
- Overgrowth and psychosyndrome

In the case of an inherited chromosomal aberration chromosome studies in members of the family may help to prevent the birth of malformed children. Empirical-risk data are available with regard to the relationship between maternal age and specific chromosomal aberrations.

Long-term administration of cytostatic drugs or the application of X-rays particularly around conception may also be an indication for chromosomal analysis. The application of modern techniques of chromosome banding is necessary for the detection of more subtle structural defects. Finally, chromosome studies can help to elucidate changes in the rate of chromosomal aberrations in special risk groups in comparison with the general population (census study).

D. Methods and results of human epidemiology

The main object of epidemiological studies is the detection of causal associations between aetiological factors and congenital malformations in human populations. These investigations are performed by two different approaches: the retrospective and the prospective inquiries.

1. *The retrospective inquiry* starts with the birth of a malformed child. The history of the mother is examined and unusual events which occurred during the pregnancy are investigated to assess their possible aetiological relation to the malformation. Such inquiries performed systematically on large samples may point to a common factor and may reveal a particular

agent to be a teratogen. In addition, experimental studies may assist in confirming its implication.

The retrospective inquiries have already yielded a number of valuable positive results. GREGG (1941) and SWAN et al. (1943) showed in this way that rubella was responsible for malformations which hitherto had been considered to be hereditary. In 1961, LENZ in Germany and MCBRIDE in Australia reported a possible connection between the use of thalidomide and the increase in certain previously rare malformations concerning chiefly the limbs in newborn babies. Animal experimentation supported this and statistical analysis performed on large samples of the population showed a clear correlation between the increase in the incidence of severe limb malformations and the use of thalidomide between 1959 and 1962.

2. *The prospective method* excludes the element of bias inherent in retrospective studies. The inquiries begin with gestation; every drug prescription and possible infections are recorded during the pregnancy, which makes the collected data more objective. But it is difficult to pursue them: enormous numbers of cases must be examined before a reasonable number of malformations for statistical treatment is encountered. For instance, in order to get information about 100 spinae bifidae, the investigation should comprise more than 100,000 pregnancies. Therefore, such prospective studies must be undertaken on a national multiregional scale in a number of countries.

For instance, a recent survey in Scotland showed that during pregnancy, over 97% of 1,369 women had taken prescribed drugs and 65% unprescribed drugs (NELSON and FORFAR, 1971). It was found that significantly more of the mothers who gave birth to children with congenital malformations had taken drugs than mothers in the control group.

A prospective epidemiological study by the French Ministry (SPIRA et al., 1973) on 20,000 women examined in the 3rd month of pregnancy and whose children had been observed at birth and one year later, yielded the following results: 9,566 women had taken various sex hormones (progesterone, progestogens, and synthetic oestrogens). They were compared with a control group of 8,387 women who had taken no drugs. The statistical analysis was made on the entire group of sex-hormone-treated women, and on individual groups who took only progesterone compounds, oestrogen compounds, or a combination of oestrogens and progestogens.

In each of these groups the percentage of congenital malformations was different from that found in women who had not taken sex hormones during their pregnancy.

DEGENHARDT and KOLLER (1973) have analysed a prospective multiregional investigation which was initiated in 1963 by the "Deutsche Forschungsgemeinschaft". Of 5,800 pregnant women, 1,098 (18.9%) did not use any drug during the first trimester of pregnancy. For evaluation, 61 subgroups were distinguished according to the drugs mostly used during the first trimester of pregnancy. Phenacetin, tranquillizers, and five single drugs have been chosen for special analysis: they are acetylsalicylic acid, ethyl-

phenylephrine, meclizine, diazepam, and a combination product containing progestogen and oestrogen.

Until now, no association has become apparent and these results are being reassessed in a larger sample of pregnant women. Such studies should lead to a better knowledge of the teratogenic danger of drugs in human populations.

III. CHARACTERISTICS OF MAMMALIAN DEVELOPMENT

The development of the conceptus consists of two fundamental processes: cell multiplication and differentiation. In the embryonic stages, the cells multiply actively and produce inductors which mediate the morphogenesis of the organs. Hence, in the first stages of development, the physiology of the embryo is, in fact, cellular. The true physiology can be defined in the foetal stage when the organs are sufficiently differentiated to perform a number of vital functions (Table 4).

This development depends on the continuous supply of nutrients which are transferred from the mother across the placenta. In mammals, digestion, respiration and thermogenesis are almost entirely controlled by the maternal organism, and the prenatal life is thus dominated by the placental function.

A. Prenatal physiology

During the prenatal development, two main stages can be distinguished: the embryonic phase, which occupies the first two months, and the foetal period from the third month to the end of pregnancy.

1. *The embryonic period:* this stage is characterized by cellular events, mainly proliferation and migration.

- From fecundation to the end of gastrulation, the embryo consists essentially of a population of dividing cells of similar appearance. Disturbances at this stage will lead to death while teratogenic effects are rare.
- The period of the great cellular movements occurs at the stage of implantation and placentation of the egg. These movements are determined by selective mitotic stimulations in cell groups having already a prospective tissular differentiation. They result in differential growth and modelling.

The determinants of this stimulation are protein inducers, so that all the factors interfering with protein synthesis have a marked teratogenic effect at this stage through an impairment of the sequence of the normal induction mechanism.

The general metabolic functions are characterized by a predominance of anaerobic glycolysis of carbohydrate metabolism, the pentose pathway being very active. Specific metabolic functions commence activity within the cells.

2. *The foetal period* is mainly characterized by the differentiation of the anlagen into definite organs, the general growth, and the storage of energy substrates. At this stage the great morphogenetic events are over and the susceptibility of the conceptus to teratogenic agents greatly decreases. The important processes are the induction of new specific enzymes in the developing organs. If disturbances occur at this stage of maturation, they are unlikely to have any morphological effects and may be harmless to the foetus

Table 4

Period of cleavage	All cells of similar appearance and mitotic activity.	One to n cell stages in oviduct. Morula, early blastocyst in uterus.	
Period of determination	Prospective significance of cell groups, "selective" differences in mitotic activity. Polarization of blastocyst. Beginning of "chemo-differentiation"	Blastocyst	Implantation Placentation
Period of differentiation	Histo- and organogenesis. Further "chemo-differentiation"	Embryo	Junction to maternal blood circulation
	Auxano-differentiation. Organs assume "normal" shape and function.	Foetus	

since most organs are not functional and do not use their specific enzyme assortment.

The foetal physiology is more simple than that of the neonate. The central nervous system does not play an important role; normal development can even be observed in decapitated foetuses. For the most part, excretion is performed by the placenta; nevertheless, the kidneys function very early and it is possible to find urine in the bladder of human foetuses from the third month of gestation. The circulation is regulated at an early stage by adaptative processes: hypoxia produces acceleration of heart rate in the lamb foetus at mid-gestation; vagal bradycardia reflexes function about the 60th day.

Since the important physiological functions are performed by the maternal organism, the energy requirements of the foetus are low. It seems that all the metabolic reactions proceed under low oxygen tension. The oxygen uptake of the foetal lamb is 4 to 5 ml/kg/min while it is 12 ml/kg/min in the newborn.

3. *The newborn period* is characterized by the adaptation to extra-uterine life. It is important as a test period in which metabolic anomalies acquired in the course of gestation may cause a dysfunction of the enzymatic systems required for the autonomic life of the newly born.

The general metabolism performs a switch-over *from an anabolic situation to a catabolic one.* Before birth, the metabolic flow was oriented from the mother to the foetus, which stored glycogen and lipids. After birth new needs of energy arise for thermogenesis, respiration and motility. This energy is supplied by utilization of stored glycogen and lipids.

Interference with storage or utilization processes may have a deleterious effect on the survival of the newborn.

To sum up, the foetal physiology is dominated by growth and storage processes; the most important functions are performed by the placenta.

B. The placenta and the nutrition of the embryo

1. *The placenta* mediates the attachment of the embryo to the uterine wall and displays a more or less intimate intricacy of maternal and foetal tissues in different mammalian species. In man, and Insectivora, the placenta is haemochorial; the chorionic villi are directly in contact with the maternal blood. In Chiroptera and Carnivora the placenta is endotheliochorial: the chorion is separated from the maternal blood by the endothelium of the uterine vessels. In cattle it is syndesmochorial: the chorion is not directly in contact with the maternal vessels; there is an intermediate layer of connective tissues. In some species (pig, horse, monkey) the epithelium of the uterine wall is intact and the chorion is adjacent to it: the placenta is epitheliochorial.

Whatever its structure the placenta has the same essential role in the transfer of molecules in both directions. An impairment of the placental function can be a limiting factor for foetal nutrition.

Moreover, the placenta synthesizes hormones. It performs a great number of enzymatic activities directed towards detoxifying processes: oxidation, reduction, conjugation and hydrolysis.

The early stages of the formation of the placenta, its permeability and its detoxification activity need further study. Most of the investigations performed so far relate to later stages which are less important in teratology. The nutritional role is first performed by the yolk-sac membrane: its modification by trypan blue or kidney antisera has been suspected to be the cause of teratogenic effects.

The transfer of drugs across the placenta may be considered a particular case of transfer across a biological membrane analogous to the intestinal wall. If a drug is adequately absorbed after oral administration and distributed in the tissues it may be capable of crossing the placenta.

The different processes involved are the following (Fig. 3):

– *Active transport* requires a particular structure of the molecule to fit with the specific membrane carrier (Scheme 1). Very few drugs possess this specificity. In this case a competitive effect between molecules of related structures is observed.

– *Passage through membrane pores* cannot be achieved by molecules with a molecular weight higher than 100, which is the case for the great majority of drugs.

– *Pinocytosis* may account for the passage of very small quantities of macromolecules, especially viruses and immunologically active substances.

– The most important process for the transfer of most compounds through the placenta is *the simple diffusion process*.

In fact, the chief characteristics of the passage through the placenta for most drugs are in keeping with the general laws of diffusion through a lipoprotein membrane. The rate of diffusion is proportional to the concentration of the drug; there is no saturation effect, no energy requirement, and no competition between related molecules.

Considering the fundamental properties of a lipid membrane it can be predicted that the rate of diffusion of a compound through the placenta will depend mostly on its lipid/water partition and also on its degree of ionization.

Concerning the *lipid/water ratio*, it has been observed, in fact, that lipophilic drugs pass readily into the foetal circulation. It has been shown experimentally that the lipophilic oestrogens cross the placenta in contrast to their hydrophilic glucuronides, which are hardly able to pass the membranes.

On the other hand, *the degree* of ionization of the drug at a physiological pH must be considered. The non-ionized form of the molecule diffuses but completely ionized compounds penetrate very slowly through the placenta. For instance, strongly quaternary bases used as polarizing muscle relaxants can be detected in the foetus only if several hundred times the pharmacological doses are administered.

The majority of drugs are, in fact, moderately lipophilic and only weakly basic or acidic so that they have little difficulty in crossing the placenta.

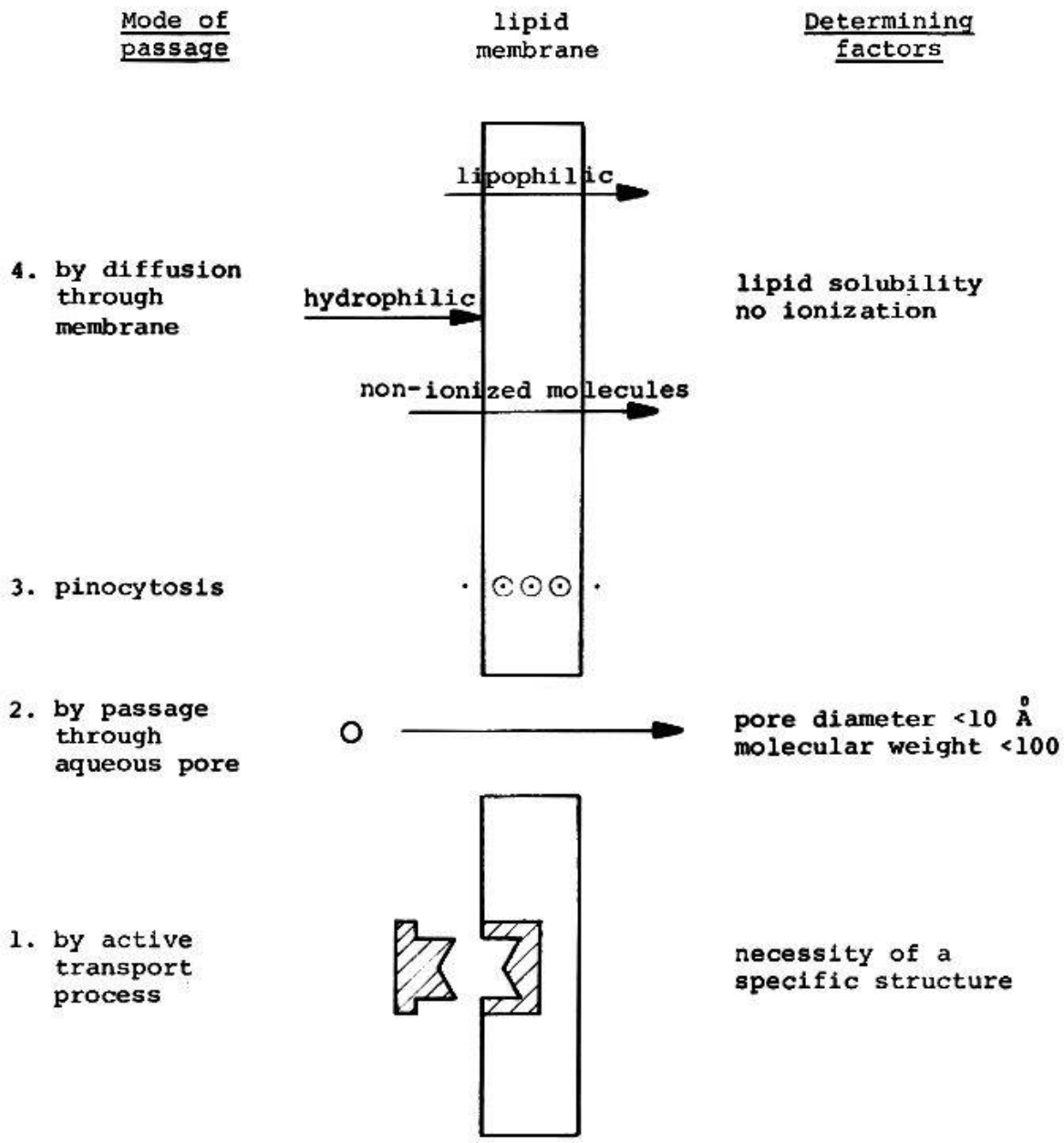


Fig. 3. Placental transfer of various compounds.

In addition to the rate of passage through the placenta several factors may affect the concentration of the drug in the foetus.

The degree of binding to plasma proteins: salicylate was found to bind *in vitro* to a greater extent to maternal than to foetal plasma. *In vivo*, foetal blood levels of sulphonamide have been found to be 10% to 30% below the maternal level, perhaps owing to differences in protein-binding capacity.

The pH of the blood is different in mother and foetus and this could give rise to an unequal concentration of ionizable drug. The fact that the level of pethidine in newborn infants is higher than in the mother has been attributed to the lower pH of the neonatal blood.

The destruction or binding of a compound in the placenta may also limit its concentration in the foetus. This limiting factor is important for endogenous

catecholamines, hydroxytryptamine and tyramine, but it does not apply to drugs in general.

The distribution of the drug throughout the body. Some drugs are known to show specific affinity for certain organs or tissues. Whole-body autoradiography of the foetus in experimental assays serves to compare the disposition of the drug in the foetus with that in the parent animal.

The metabolism and excretion of a compound are the factors which limit the duration of its action. The fact that many enzymes of drug metabolism are absent from the foetal liver may constitute a potential mechanism of protection. In the case of an even tissue distribution, and as soon as the concentration of the compound decreases in the maternal blood, there is a reversal of the diffusion through the placenta and the compound contained in the foetus passes back into the mother's blood. However, if a lipophilic compound were metabolized into its hydrophilic metabolites in the foetus, these metabolites would not cross back through the placenta and could remain in the foetus.

This situation does not hold good after birth because of the absence or low activity of oxidative and glucuronide-forming enzyme in the newly born. Compounds retained during pregnancy may remain for a long time in the newborn and may produce toxic effects. An example is provided by chloramphenicol, which, in addition to being retained, enters into competition with the excretion of bilirubin.

Generally speaking, the fact that a compound crosses the placenta does not mean that it will have an embryotoxic or teratogenic potential. The nature of the compound or of its metabolites and the possibility of their accumulation in the foetus are very important.

Although the study of placental transfer mechanisms is of great interest, it seems that the nature of these phenomena is only of limited relevance to the manifestation of embryotoxicity or teratogenicity of absorbed compounds.

2. The nutrition of the embryo

The mammalian embryo, being practically devoid of nutritional stores, is highly dependent on the maternal organism supplying nutrients via the placenta. The nutrition of the mother is thus particularly important since nutritional deficiencies may cause embryonic death, malformations, and stunted foetuses.

The nutritional requirements of the mother are increased during pregnancy: some parts of the nutrients are utilized for the growth of the foetus and others for energy storage. Placental hormones with a growth-hormone- and prolactin-like activity produce a shift to a state of anabolism in the maternal organism characterized by the storage of free amino acids and peptides, minerals, and other substances. This pool of nutrients is reutilized during the suckling period.

Both the quantity and the balance of the different components of the mother's diet may affect the embryo.

Concerning the quantitative requirements, severe overall restrictions in the diet, occurring during the embryonic period, may result in the death of the conceptus. When they occur during the foetal period, its growth is impeded. Sensitivity to hypocaloric diets varies greatly from species to species: whilst cows and rabbits are not very sensitive, rats show a marked sensitivity; a 50% decrease in the diet causes sterility and a hypocaloric diet may increase the teratogenic action, e.g. of cortisone.

Low-Protein diets alter the growth during the foetal period. They are more noxious in the course of the embryonic period, causing disturbances in pituitary and ovarian function so that the uterine mucosa is no longer adapted to the implantation and nutrition of the young embryo, which cannot be maintained. Moreover, deficiencies in one of the essential amino acids may result in abnormalities, resorptions, and stillbirth: the essential processes of morphogenesis are in fact very sensitive to the supply of amino acids. The optimal balance in amino acids is found in protein of animal origin, not in vegetal protein.

In the rat, deficiencies in *essential fatty acids* such as linoleic acid result in sterility and abortion.

Carbohydrates represent the first source of energy for the embryo and foetus: glucose and fructose are the sugars most readily used. Some organ systems, such as the nervous system, are more sensitive than others – the heart for instance – to carbohydrate deficiencies.

Inorganic substances are also important for the development of the embryo. Some are essential as enzymatic constituents. Zinc deficiency is associated with important malformations in the rat. Other elements are important components of intercellular substances, such as sodium in connective tissues and calcium, phosphorus and manganese in bone. In these cases deficiencies do not cause true malformations: bone curvatures due to defective calcification are observed.

Vitamins readily cross the placenta and are largely utilized by the foetus. If the mother's supply is not increased during pregnancy, the normal level of vitamin in the mother's blood decreases progressively and is often very low at the end of the gestation. Malformations through vitamin deficiencies have not been reported in the human, except for folic acid. A lack of vitamin B_2, vitamin A, pantothenic acid, folic acid, or vitamin B_{12}, is markedly teratogenic in the rat and in other animal species. Deficiencies of other vitamins, such as ascorbic acid, nicotinic acid, thiamine, biotine, and vitamin K have not proved teratogenic. An excess of some vitamins, such as vitamin A, may also disturb the prenatal development in rats, mice, rabbits and guinea-pigs.

IV. EXPERIMENTAL TERATOLOGY

A. Basic principles

The basic principles of teratogenic drug testing are similar to those underlying the detection of toxic reactions in adults. Therefore drug teratogenicity can be considered an aspect of general toxicology. However the actions of drugs on the embryo are more complex than those on the adult organism. In teratogenesis one is dealing with two biological systems – the pregnant female, and the embryo – the specific reactions of which can be completely different. Consequently a drug that is non-toxic to the female may still be apt to kill the embryo or produce congenital malformations. The problem is even more complex since the influence of the placental transfer of drugs and their metabolic fate in the embryo are only partially understood. To compete with the various mechanisms which control prenatal development, very complex conditions have to be fulfilled.

The action of a teratogenic agent on the conceptus depends mainly on three conditions: (1) the developmental stage of the embryo, (2) the genetic susceptibility of the embryo, and (3) the physiological or pathological status of the mother.

1. The developmental stages

The period in which injurious agents mainly affect the development of the human embryo is very short. It is largely completed by the 8th week of pregnancy, just about the time when a women knows that she is pregnant (Fig. 4).

During the pre-implantation period, when the blastocyst lies free within the uterus and depends for its nutrition on the uterine secretions, exogenous agents can kill the embryo, but there is no evidence that they can produce congenital malformations. This is the period of maximal embryolethality. However, minor injuries can be overcome without manifest harmful consequences on the growing embryo because, during the segmentation stage, many blastomeres retain their totipotency, being able to replace damaged cells by newly formed cells. Once implantation has occurred in the human, 7–8 days after fertilization, the embryo undergoes very rapid and important transformations.

The sequence of embryonic events shows that each organ and each system undergoes a critical stage of differentiation at a precise moment of the prenatal development. It is during this critical period that the vulnerability of the developing embryo is greatest and that specific gross malformations can be produced. *This is the susceptible period*, which lasts until the 56th day in the human embryo. *The foetal period* begins at the end of the 8th week, when little further differentiation of organs remains to be completed. The most important events at this stage are the closure of the palate, the reduction of the umbilical hernia at the end of the 9th week, the differentiation of the external genitalia, as well as the histogenesis of the central nervous system.

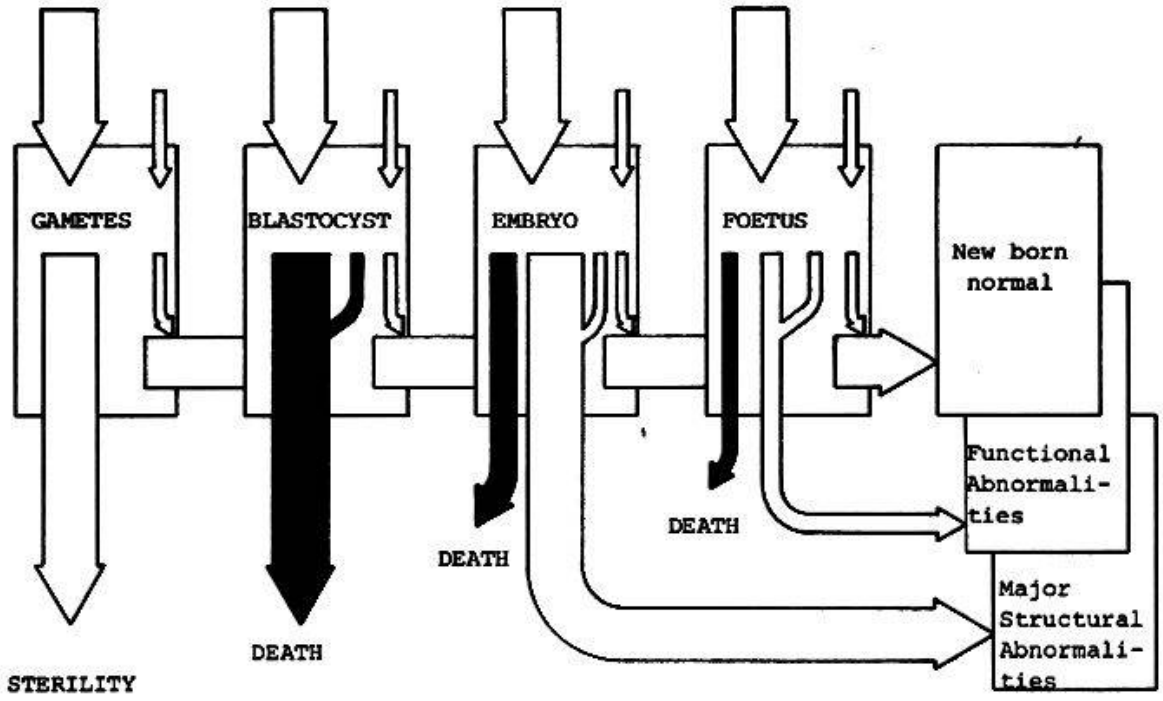


Fig. 4. Schematic representation of the influence of teratogenic factors on gametogenesis and various stages of prenatal development. Strong teratogenic agents: wide arrows; weak teratogenic agents: narrow arrows.

This latter process lasts for the entire period of intra-uterine development and is not complete until several months after birth. Consequently, during the foetal period, teratogenic agents do not produce major morphological malformations but can impair the differentiation of external genitalia, leading, in severe cases, to pseudohermaphroditism. Interference with the histogenesis of the central nervous system can lead to various degrees of encephalopathy.

2. Genetic susceptibility and species differences

The reaction of the embryo to exogenous agents depends upon its genetic constitution. The reaction of the embryo to a specific compound varies not only between different animal species, but also within a given species, between each strain, and even between individuals of the same strain (Figs. 5 and 6 may serve to illustrate the differences in the critical stages of embryogenesis between two species of laboratory animals).

Cortisone, a potent teratogenic agent in the rabbit and in the mouse, does not produce malformations in the rat. Thalidomide, which produces obvious malformations in the rabbit, is apparently innocuous in the rat. A purine analogue, azathioprine, which is highly teratogenic in the rabbit and the mouse, does not produce anomalies in the rat. Teratogenic susceptibility differences in strains have been demonstratcd. For example, the same regimen of treatment with cortisone causes 18% malformations in C57 BL mice while in the A/Jax strain 100% malformations are produced.

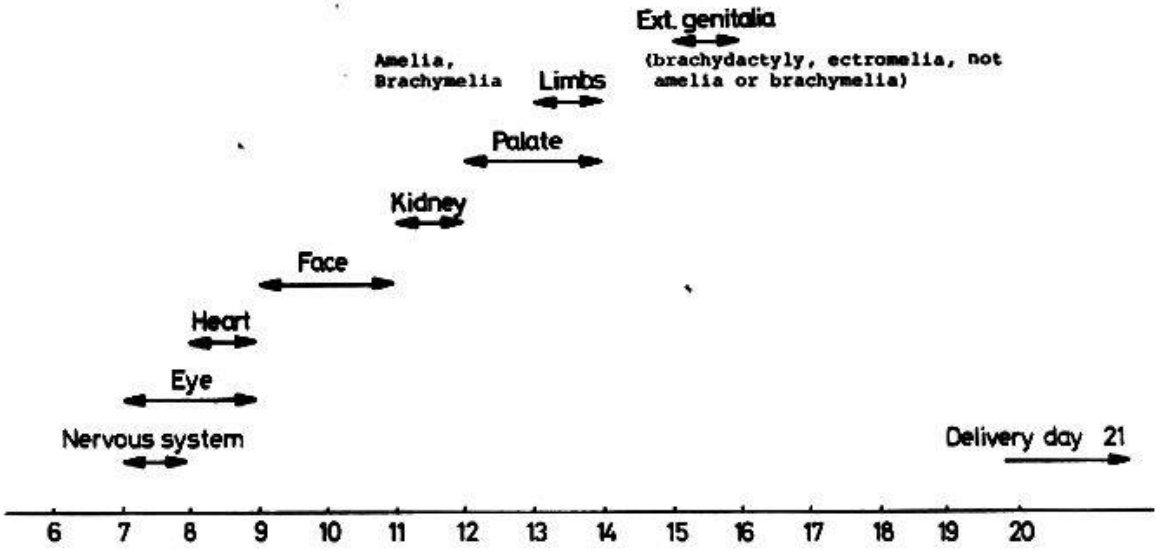


Fig. 5. Critical periods in embryogenesis of the rat.

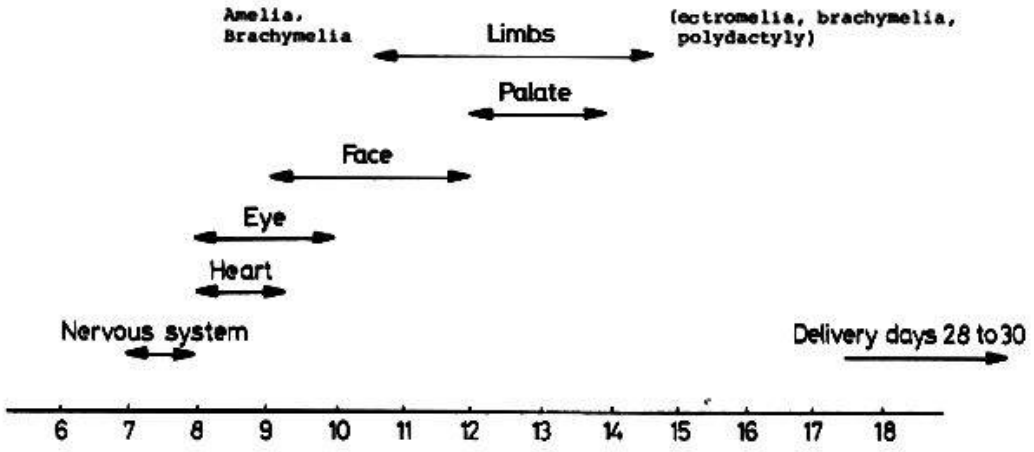


Fig. 6. Critical periods in embryogenesis of the rabbit.

3. Physiological or pathological status of the mother

Besides the developmental stage and the genetic constitution of the embryo, the action of a drug is dependent on the physiological and pathological condition of the mother. The most important physiological factors are: age, diet, hormonal balances, and uterine environment. Among pathological conditions, metabolic diseases, diabetes and obesity tend to increase the action of potential teratogenic agents.

B. Teratogenicity testing

1. The experimental procedures

In the determination of the toxicological potential of a drug, the choice of the conditions of treatment, that is to say, the route of administration, the doses, the number of animals per dose, the duration of treatment, etc., is of major importance.

The problem is particularly difficult in teratology because of the complex nature of the biological system that is involved (see chapter III).

In addition, it must be noted that the treatment may also, under certain conditions, (excessive doses, local irritating effect at the site of injection, etc.), induce in the mother pathological changes which by themselves are incompatible with the survival and the normal development of the embryo: such conditions should be avoided, since abnormalities observed in the foetus would be devoid of any specificity.

The toxicity of the compound for the embryo may manifest itself either by the death of the litter or by the appearance of malformations. A compound may cause embryolethality without being necessarily teratogenic; but with some drugs, these two toxic effects are associated and, in such a case, the early death of the embryo, followed by resorption, may hide a teratogenic effect: the teratogenic activity may then be detected only in a rather narrow range of doses, at which embryolethality remains still moderate.

The sensitivity of the embryo to the toxic effects of a compound depends on the developmental stage reached by the embryo when it is submitted to the action of the latter. One may consider that the stage of embryogenesis properly speaking (or organogenesis stage), which begins in most species at the time of blastocyst implantation and takes place between the blastogenesis stage and the foetal development phase (or foetogenesis phase), corresponds to the period of sensitivity to teratogenic effects. If a toxic injury manifests itself during the formation of the blastocyst, there are, it appears, with very rare exceptions, only two possibilities: the blastocyst dies, or it overcomes the injury, the consequences of which for the subsequent development of the embryo are then very limited or non-existent.

Furthermore some authors suggest that if virgin females of the rodent species are used, and if the treatment is begun after the implantation, which has the advantage of eliminating a possible effect of the compound on the implantation process, it is easier to check, *a posteriori*, the pregnant state by detecting the implantation sites when sacrificing the mothers before delivery.

Although in teratological studies, as in any type of toxicological study, the investigator must be allowed to adjust his protocol to the particular case of the compound under study, and not be curtailed by too rigid rules, it is possible to propose some general recommendations for the conditions of treatment. First of all, the state of health and standards of care of the animals must be carefully controlled. Animals that are to be used for teratological studies of drugs must be healthy and should be housed under the best possible environmental conditions. The best practices of animal care must be maintained; specified pathogen-free animals are not necessarily required under these conditions. It is advisable that strains with known genetically unstable constitutions be avoided. The animal quarters should provide constant temperature, adequate light and protection from noise or other interference. When temperature and light cannot be controlled, the seasonal variation of the reproductive activity of the animals must be considered.

Caging should conform to the best standards available. The use of pesticides must be avoided. It must be remembered, particularly with drugs that act upon the central nervous system, that housing groups of animals in a single cage may increase the noxious effects of certain drugs (group toxicity). All animals should receive an adequate diet; since the administration of a drug may decrease the food consumption of the treated animals, it may be necessary to adjust the food intake of control animals correspondingly.

a) Number of species

For routine teratogenic drug screening animals with a high fertility rate and a short duration of pregnancy should be used. At least two species, one rodent species, the rat or the mouse, and one lagomorph, the rabbit, will fulfil the general requirements.

b) Choice of route and method of administration

Generally the drug should be administered by the route intended to be used in man.

In the case of the oral route, one must determine (preferentially by blood-level determinations) whether the compound is absorbed by the species under study at the level of the gastro-intestinal tract. If the compound is insoluble in the chosen vehicle, its particle size must be sufficiently small and well defined, in view of the role this parameter plays in the digestive absorption.

The compound may be administered in the food on the strict condition that its stability in the food has been ascertained. The amount of the compound ingested will be estimated on the basis of the daily food intake. In the view of some authors this method of administration might have the advantage of covering all the developmental stages by more or less consistent exposure of the target organs to the compound and/or to its metabolites. However, the administration of the compound by stomach tube (either as a solution or a suspension in the proper vehicle), particularly in the mouse and the rat, or in capsules (in larger animals), seems to be more frequently used. It has the advantage of allowing the amount of the compound ingested to be accurately determined and usually ensures high levels of absorption.

The use of the intravenous, intramuscular, or subcutaneous routes is generally without problem but the interpretation of the results may be difficult, when the local tolerance at the site of injection is poor. It appears preferable to avoid the intraperitoneal route, because of the risk of accidental injection at the level of the uterus and of the effects of a possibly poor local tolerance.

Although it may be conceivable to study the teratogenic effect of a compound administered by the rectal or vaginal routes or by inhalation, the use of these routes is fraught with difficulties, especially in the smaller animal species (problem of checking that the suppository remains in the rectum; risk of abortion by vaginal stimulation; hypoxia and stress caused by some inhalation procedures).

In the case of a compound to be used in man by one of these routes, it appears possible to take into consideration the results of an experiment performed by the oral or, better, by the intravenous, intramuscular or subcutaneous routes. A prior comparative study of the metabolism of the compound in the animal species used, administered by the route intended for therapeutic use and by the route chosen for the experimental study, will generally justify the decision taken.

For compounds to be used for dermal, nasal, auricular, or ocular treatments, a study by any route that is demonstrated to result in systemic exposure may be adequate.

Finally, during the course of treatment females should not be subjected to any kind of rough handling, which by itself could cause disturbances of the pregnancy.

c) Period and duration of treatment

As a general rule, the treatment must begin immediately after implantation and continue during the whole period of organogenesis. In experiments using rodents, for instance, the following periods appear to be adequate:

- in the mouse and the rat, from the 6th day to the 15th day inclusive, day 0 being marked in the mouse by the presence of a vaginal plug, and in the rat by the presence of spermatozoa in vaginal smears;
- in the rabbit, from the 6th day to the 18th day inclusive, 0 day being that of mating.

Nevertheless, in a number of cases, e.g. severe impairment of the health of the mother animal caused by excessive pharmacological activity, tolerance developing to the compound tested owing to the induction of metabolizing enzyme, etc., it may be useful or even necessary to limit the treatment of the pregnant females to a few days or even a single day, provided the whole period of organogenesis is covered by using an appropriate number of animal groups.

Furthermore, the pharmacokinetic characteristics of the compound in the animal species that is tested should be taken into account. In the case of a compound with a short half-life, for instance, administration of the compound twice a day during the whole period of organogenesis may be envisaged. Alternatively, in the case of a compound with a long half-life period, slow release or depot preparations in particular, intervals of several days between each consecutive administration may be appropriate.

d) Choice of doses

The selection of adequate doses is most important.

In the case of drugs the factors to be taken into account are the following:

- the nature of the pharmacological activity exerted in the particular species and the dose at which this activity manifests itself
- the therapeutic dose of the compound in man

- pharmacokinetics and metabolism in the animal species in comparison to man
- the toxicity of the drug to the mother animal

e) High dose effects

Since the aim of toxicity studies in general is to detect untoward effects and to demonstrate how these effects are related to the dose, high doses up to the maximum tolerated dose are commonly administered.

This procedure has its limitations in the case of certain drugs such as hypnotics, local anaesthetics, hypotensives and diuretics, for instance, which, owing to excessive pharmacological activity, may impair the general condition of the animal including metabolism, circulation, nutrition and temperature regulation, secondarily leading to organ damage. The developing foetus is particularly sensitive to this kind of disturbance and early foetal death, resorptions, malformations, or reduction of growth may occur.

Very high doses which might impair the general health of the pregnant animal can produce malnutrition of the embryo and consequently the appearance of an unspecific teratogenic effect: such an effect, although presenting difficulties in the interpretation of the results of the teratological study, would have no significance with respect to the normal conditions of use of the drug in clinical practice. Furthermore, by administering excessively high doses, without any relation to the pharmacological doses, the metabolism of the compound may be modified quantitatively as well as qualitatively.

With these considerations in mind it appears desirable that the highest dose studied should exert in the pregnant female some systemic effect, for instance, a slight impairment of the weight increase in comparison with control animals. It is important that the highest dose does not cause excessive embryolethality and the foetal loss should not be higher than 50%.

Teratogenic effects produced by extremely high doses, in comparison with the human intake, demand special consideration. In general, they are considered relevant only if these doses did not impair the well-being of the mothers and if they were not dose-dependently accompanied by other disturbances of the pregnancy.

f) Preliminary investigations and final experiments

The choice of the maximum tolerated dose makes it desirable to perform a preliminary experiment on a few pregnant females, with 2 to 3 doses and a limited number of animals per dose.

Such a study is considered necessary because the pregnant female is usually more susceptible to the general toxic activity of a compound than the normal female.

In the final experiment, one should add to the highest dose lower dose levels. In order to establish a dose-response relationship generally two doses are chosen on a logarithmic scale: the lowest dose, which must not cause any embryolethality, will approach the therapeutic dose and is usually chosen

to be twice or three times higher than the dose (per kg of body weight) intended for use in man.

A series of control animals will be treated with the vehicle, under the same conditions as the animals receiving the compound under study. The number of animals per series must be sufficient to enable a statistical evaluation of the results to be made (cf. Chapter IV, B 4).

g) Choice of the animal species

Rodents. A large variety of malformations can be experimentally induced in rodents. In the mouse the most frequent gross malformations involve the palate, the central nervous system and the tail. In the rat, malformations of the eye and skeletal deformities may predominate.

In lagomorphs, eye malformations are quite exceptional whereas coelosomia and central nervous system and skeletal anomalies have been among those most frequently observed. However it must be emphasized that there are no general rules. Usually there is a relationship between the morphological type of the anomaly and the time of drug treatment.

Rat. Often considered as a reference animal, the rat has several advantages over other species: short duration of pregnancy, high fertility rate, large litters, and relative resistance to the toxic effects of many compounds. Rats also have a fairly good developmental stability. In the Wistar line the spontaneous rate of gross malformations is approximately one per 1,000 foetuses. The main limitation of the rat is its poor teratogenic susceptibility to some drugs, like cortisone, thalidomide, and azathioprine, for example. It is therefore not recommended to draw definite conclusions on the basis of results obtained only in this species.

Mouse. This species shares the advantages of the rat and in addition, mice are particularly susceptible to some teratogens. In Swiss albino mice the spontaneous malformation rate is approximately 0.5%. The resorption rates are also higher than in the rat. Therefore it may be necessary to have a large control group.

Other rodents have been used only rarely for teratogenic investigations.

The few teratological investigations performed *in hamsters* have given satisfactory results comparable with those obtained in rats and mice. However, from the available data it does not appear that this species offers any advantage for teratogenic drug testing.

In *guinea-pigs* pregnancy lasts three times as long as in rats and litters contain only 2–4 foetuses. It is desirable to obtain more information on this species.

Lagomorpha. The discovery of the susceptibility of rabbits to thalidomide led many biologists to consider them as one of the most favourable animals for teratogenic studies. The use of the rabbit has also been advocated as a non-rodent species.

The pig. The possibility of using the common domestic pig (Sus scrofa) for teratogenic screening has recently been explored. The pig is far removed in

evolutionary descent from rodents and could therefore fulfil official requirements of a non-rodent representative. The chief advantages of the pig as compared to other non-rodents are that it is easily available and highly prolific (average litter 10–11). Oestrus occurs at 21-day intervals and mating can take place at 7–8 months of age. The gestation period is about 115 days, which is of some advantage when compared with the rhesus monkey, which has a gestation time of 164 days. The embryology and the genetics of the pig are fairly well known. The incidence of spontaneous malformation is relatively low. The susceptibility of pig embryos to teratogenic drugs seems to be fairly high; vitamin A induces teratogenic effects and thalidomide causes a high incidence of visceral, facial and urogenital malformations.

In experimental practice, however, the use of the pig is much limited. The expenditure of care, including the large amount of space and the comparatively enormous amounts of compound required to perform toxicological and teratological studies, constitute real handicaps. It was hoped that the problem of size would be overcome by the use of a miniature breed of pigs, but minipigs are not readily available and their fertility and prolificacy are low. More experience is needed before a statement can be made as to the usefulness of the pig in teratological investigations.

The dog. Although polytocous like rodents, dogs have a recurrent oestrus at intervals of about 6–8 months and even longer when maintained under laboratory conditions. Induction of ovulation by hormonal stimulation is not advisable owing to the possibility of drug interaction. The limited period of heat and the long gestational period require a considerable amount of time to perform teratogenic studies. Further, the interpretation of the experimental results requires data on the normal incidence and type of malformation for the particular breed used.

As far as the teratogenic susceptibility of dogs is concerned it is difficult to make a definitive statement because only limited data are available. The critical period of organogenesis seems to take place between days 12–27.

The cat. Very few data are available for this species, which is rather difficult to maintain and breed under laboratory conditions. The most favourable period for drug-induced teratogenicity seems to be between days 12–27 of gestation. Particularly the difficulties encountered in breeding are against the use of cats for routine teratogenicity testing.

Primates. The phylogenetic proximity of monkeys and human beings and the great similarity of reproductive physiology in women and certain monkeys, like the rhesus, led to the suggestion that this species might be more reliable for teratogenic drug testing than other laboratory animals.

Wilson (1971) proposed a teratological test using 25–30 pregnant monkeys. He recommended the following procedure to find doses and treatment times causing embryotoxicity. A preliminary screen is conducted with 15 animals, one treated at each of three dose levels at five gestational stages, followed by hysterotomy on day 100. Larger doses are used if no embryotoxicity (growth retardation, intra-uterine death, or malformation) is observed

initially. Additional animals are then treated with doses and at times associated with embryotoxicity, to define these variables better, and some pregnancies are allowed to go to term if needed.

For a general appraisal of the possible usefulness of monkeys in teratogenic testing, the main results obtained in recent years need to be confirmed. Since several species of macaque monkeys and baboons react to thalidomide in the same way as man, it has been assumed that simian primates would be better and safer test animals for the evaluation of drugs than the conventional laboratory animals.

This assumption has not been substantiated by screening various drugs in rhesus monkeys. From his personal experience WILSON (1973) concluded "it must be emphasized that the close parallel between man and monkey as regards the teratogenicity of thalidomide has not been demonstrated for other drugs except those with androgenic properties".

Several drugs which are teratogenic in rodents, like meclizine, 6-aminonicotinamide, acetazolamide, methotrexate, etc. did not induce malformations in monkeys.

An interesting case is that of methotrexate, a folate antagonist, which is highly teratogenic in rats at 0.3 mg/kg (WILSON, 1970). In rhesus monkeys no teratogenic effects were observed with 3 mg/kg and even higher doses.

From his comparative observations WILSON (1971) concluded that rats are teratologically more susceptible to acetazolamide, retinoic acid, methotrexate, 5-fluorouacil, hydroxyurea and vincristine. Conversely, monkeys were particularly susceptible to thalidomide. The reason for these species differences is still not clear. It was suggested that it might be related to differences in the metabolism of test substances. However for a compound like methotrexate the available data on distribution and excretion do not reveal differences between rats and monkeys that could explain the differences in embryotoxicity. As pointed out by WILSON (1971) these data raise the question which of these two species is more appropriate for the teratological testing of drugs in general.

2. Particular problems raised by certain drugs

The experimental difficulties encountered in routine teratogenic drug screening include some problems inherent in the action of certain types of drug, for example:

Compounds which destroy the intestinal flora, as do certain antibiotics in the rabbit; drugs with a prolonged, intensive pharmacological activity; compounds causing enzymatic induction; compounds which rapidly harm the health of the mother and can be administered only for a short period of time. To overcome these difficulties, the treatment may be limited (cf. Chapter IV, 1c).

Psycho-active drugs

Substances which are capable of modifying the pituitary-hypothalamic system and of causing release of lactogenic hormone (butyrophenones, reser-

pine, phenothiazines, etc.) are likely to result in delayed implantation. Consequently the embryos exhibit apparent growth retardation which in fact reflects only the retarded implantation. Such a reaction is not likely to occur in humans.

Similar problems are also encountered with certain steroids or pituitary hormones when tested in conventional laboratory animals.

Hormonal agents

Corticosteroids: The catabolic activity of these preparations induces weight loss in the mother animal.

The suppression of the immune response, a well-known effect of corticosteroids, lowers the resistance to infections.

Glycogen accumulation and fatty infiltration in the liver and, in some species, liver necroses are produced.

The particular properties of corticosteroids influence the state of health of the mother animal and consequently may harm the foetus. With ACTH the same problems arise owing to stimulation of the adrenal gland.

It might be useful to perform the experiments in specified pathogen-free animals.

Many of the anti-inflammatory steroids produce cleft palate in the foetuses of animals dosed during pregnancy. The mouse is the most sensitive species and practically all anti-inflammatory steroids will induce cleft palate. The rat and the rabbit are far less sensitive but respond to the more potent glucocorticoids such as dexamethasone and triamcinolone. Particularly in mice this effect is not considered to be specific. Cleft palate can be produced in this species by treating the dam with ACTH, which suggests that mice are sufficiently susceptible to be affected by still physiological quantities of corticoids released by the adrenals. By contrast, adrenal stimulation by extreme stress fails to induce cleft palate in the rat. Reduction of amniotic fluid caused by amniocentesis is sufficient to induce cleft palate in mice. Cortisone is known to produce oligohydramnios and it is suggested that the higher sensitivity of mice to cleft palate production is related to their particularly rigid craniospinal articulation resulting in less craniospinal flexibility *in utero*.

In spite of many thousands of women having received cortisone during pregnancy there is no convincing evidence that this treatment results in cleft palate or other congenital malformations in man. It appears certain that the risk of teratogenic damage must be extremely small.

Sex hormones

In rodents and in rabbits oestrogens produce a weight loss and, depending on the dosage, partial or complete resorption of the embryo. In these species oestrogens control the uterine growth and the elaboration of uterine fluids.

Progestational compounds have no adverse effects on pregnancy even at high doses, provided that administration is restricted to the period of morpho-

genesis. In later stages the gestation can be prolonged and foetal growth and parturition are delayed. This would also be true for gonadotrophins.

Simultaneous administration of progesterone and oestrogens, as in oral contraceptives, moderates the oestrogen effect. Much higher doses are necessary to cause abortions.

Anti-oestrogens will cause abortion in much the same way as oestrogens. Anti-oestrogenic compounds like clomiphene and methallibure have the same effects in rodents and are also teratogenic, probably through a pituitary-blocking action. However these effects are not likely to occur in humans because there are important physiological differences.

The information gained in rodents, which have a particular hormonal control of gestation, raises the problem of the selection of a suitable animal model for the study of the reproduction processes as they occur in humans. For instance, it has been suggested that experiments be conducted in dogs and monkeys to test the chronic toxicity of fertility-regulating agents. This recommendation can be misleading as far as the use of dogs is concerned, because this species has a particular susceptibility to some progestational compounds and presents an exceptionally high incidence of spontaneous mammary tumours.

While in rodents and in rabbits the maintenance of pregnancy requires functioning ovaries, in women, in primates, and in guinea-pigs, the placenta takes over very early the hormonal control of pregnancy. Therefore, as in women, the maintenance of gestation is not dependent on active corpora lutea but on the placental activity. In conventional laboratory animals oophorectomy causes abortion during the major period of the pregnancy. In primates, after the first six weeks, pregnancy is maintained by the large amount of chorionic gonadotrophins, progesterone and oestrogens produced by the placental tissue. By contrast, in the rabbit the production of progesterone and oestrogen in the corpus luteum is governed by the pituitary; the placenta does not produce pregnancy-maintaining hormones.

3. Criteria of congenital malformation, visceral and skeletal examination[1]

Congenital malformations can be defined as "structural" defects present at birth. They can be external, internal, grossly or only microscopically detectable. Although substances may be well tolerated by the dam they can be embryotoxic and/or teratogenic.

Embryotoxicity may be subdivided into:

- Embryolethality (post-implantation death)
- Retardation of growth and development

Retardation can consist in inhibition of the physiological growth of the foetuses or in delayed development. Inhibition of growth exists when the

[1] Although the present emphasis is placed on morphological criteria, the possibility of "metabolic malformations", i.e. enzymatic defects without structural abnormalities of the tissues should also be considered.

foetuses are normally developed but are significantly under weight. Delayed development is indicated primarily by retardation of physiological ossification of the skeleton.

An impairment of the placenta can also indirectly impair the development of the foetuses. Thus it may be advisable to examine and weigh the placenta. However, experience has shown that examination of the placenta does not offer any practically relevant information.

The following suggestions regarding the examination of foetuses for skeletal and visceral anomalies apply principally to screening studies. Therefore, priority must be given to the adequate examination of a large number of foetuses for visceral and skeletal defects.

The method(s) of examination will depend on several factors including the facilities available, the preferences of the investigator, and the size of the foetus to be examined.

External examination

Thorough external examination of the foetus in utero is a prerequisite for the success of subsequent skeletal and visceral examinations.

Large species. For larger species such as dogs, monkeys, pigs, cats, etc. the individual foetus can be examined for both skeletal and visceral defects, a procedure which is perhaps essential in view of the tendency to examine small numbers in such species. For reasons of logistics and economy, radiography of the foetus immediately after external examination is probably the most convenient method.

For skeletal examination, X-rays should be taken in at least two planes. It must be remembered that, even with quite sophisticated apparatus and the higher degree of skeletal development of large species, radiography does not provide the precision and three-dimensional flexibility afforded by alizarin staining and some expertise is required to distinguish anomalies from artefact.

Following radiography all foetuses can be examined for visceral defects and probably the simplest and yet the most efficient method is to follow a standard autopsy procedure. It is important to dissect the urinary tract and to examine the heart, particularly the interventricular septum, and the main vessels. The intact brain should be carefully removed *in toto* and sliced in order to detect internal defects.

The standard autopsy technique is most efficient when performed on freshly killed foetuses (i.e. before fixation) as the coloration of the tissues allows ready identification of organs, the flexibility of the tissues allows them to be displaced and viewed from various angles, and the patency of blood vessels can be checked either by the simple application of pressure or by injecting a coloured fluid. On fresh specimens ocular defects can be detected more readily than after fixation.

Intermediate size species. For intermediate sized species, such as the rabbit, or larger species delivered at an early stage of pregnancy, skeletal and vis-

ceral examination can be performed along the lines indicated for large species. However, instead of radiographic examination the alizarin-staining technique would appear to be the method of choice of skeletal examination.

Visceral abnormalities may be rather easily detected by examining the body cavities, i.e., the thoracic and abdominal organs *in situ* (including pelvis, uro-genital tract).

There are different methods of examination of the various topographical and organ systems; all have their advantages and disadvantages. There is no method that allows all of the foetuses to be examined for both visceral and skeletal anomalies.

Small species. Although it is possible to examine small foetuses for both skeletal and visceral anomalies in a manner similar to that used for the larger species, the process is difficult and time-consuming; it requires a combination of dissection under a stereomicroscope and the use of alizarin staining or sophisticated radiographic apparatus.

Most laboratories, therefore, circumvent the difficulties of examining smaller foetuses by increasing the group size (relative to larger species) and apportioning foetuses so that some are examined principally for visceral defects whilst others are examined principally for skeletal defects.

Of the two methods of skeletal visualization, alizarin staining is preferable. The method usually followed is that of DAWSON (1926). In evaluating the results, due account must be given to the fact that ossification of various skeletal parts is particularly active at the perinatal period (FRITZ and HESS, 1970).

Foetuses allocated for skeletal examination can also be examined to a limited extent for major visceral abnormalities. One method is to make a transverse cut across the abdomen and a longitudinal cut (on one side of the sternum) from the neck to the inguinal region; the epidermis can then be turned back as flaps and the visceral contents examined *in situ* before being removed. Alternatively, one may make one transverse cut across the abdomen and, with a pair of fine forceps inserted up through the thorax, remove the viscera intact.

Although this technique will reveal major visceral malformations and occasional minor anomalies special techniques have been developed for the analysis of visceral defects.

The most commonly used technique (WILSON, 1965) consists in cutting a series of transverse sections (0.5 to 1 mm thick), principally through the head, thorax and abdomen, which are then examined under the microscope. Fixation of the foetuses must be rapid and as well as decalcifying the foetus must leave it sufficiently firm to be sliced thinly without causing disintegration of sections. If fixatives containing picric acid (Bouin's fluid) or formalin are used it is desirable to transfer the foetuses to another preservative (e.g. ethanol) before handling and slicing them.

An alternative microdissection was proposed by BARROW and TAYLOR (1969). This technique, although adequate, may not be quite as efficient as

the Wilson technique for detecting minor heart anomalies and, moreover, tends to preclude the opportunity of performing subsequent histological examination. However, it is a useful technique for supplementary examination of foetuses principally allocated for skeletal examination.

Of the various modifications of these two techniques one that appears to be of value consists in removing the heads from all foetuses to examine them by the Wilson technique, the caracasses being apportioned to skeletal staining and/or visceral examination.

Apportioning of foetuses and recording of results

Some government agencies suggest that 50% of small foetuses be examined for skeletal defects and the remaining 50% be examined for visceral anomalies. Other authorities suggest two thirds to be subjected to skeletal staining and one third to be examined for visceral anomalies.

Preferably more foetuses should be allocated for skeletal staining, for the following reasons:

- They can be examined for major visceral abnormalities before processing.
- Differentiation or development of the skeleton (or its precursors) continues throughout gestation and general effects elicited at any time during pregnancy may be manifest as a skeletal change.

Records should be kept in such a way that abnormalities can be related to the individual foetus, the individual litter and the technique of examination. Results should be reported as the absolute proportions of foetuses affected since percentage values can be misleading, particularly when they take no account of litter effects or the occurrence of several anomalies in one foetus.

4. Number in experimentation

Numbers of animals

When rodents are used the number of animals can be large enough to satisfy statistical requirements. Although this seems reassuring it must be borne in mind that to fulfil this requirement one has to define:

- the parameter(s) to be examined, its natural incidence and frequency distribution (which determines the most appropriate method of statistical analysis).
- the magnitude of the difference from "normal" which the analysis is required to detect.
- whether the difference will be examined by a one- or a two-tailed criterion.
- the level of significance (probability) that will be acceptable as proof of a difference.
- the degree of confidence with which the level of significance will lead to the correct conclusion.

Unfortunately many of these factors cannot be defined before commencement of a study as, by definition, the parameter that is likely to be affected in screening tests is unknown.

One aspect that is known, however, is that in untreated animals major malformations occur at a very low frequency so that if they were to be the sole criterion of effect then statistical theory would demand the use of hundreds, thousands or even tens of thousands of animals in order to detect small changes in the normal rate (i.e. say doubling of a rate from 0.1% to 0.2%). Experiments of such magnitude are not feasible.

Experience has shown that screening studies involving 15–20 pregnant rodents per group (for each dose) and 3 to 4 groups (three to four doses), can provide a good indication of possible selective effects on the offspring, providing that the studies are well set up and well conducted.

For studies in which two or more methods of examination are employed (i.e. when some litters are delivered by Caesarian section, and other dams are allowed to rear their young, or when some foetuses will be examined solely for visceral anomalies and others for skeletal defects) it would be advisable to allow 10–12 litters for each method of examination.

In performing studies with group sizes of the order of 10–20 animals it is important to consider the studies as initial rather than final steps in the evaluation of safety. Also, one must be aware that effects evoking an all-or-none litter response (e.g. non-pregnancy, abortion) would need to be of the order of 40–50% before they became statistically significant. To detect effects of a lower magnitude the investigator must employ methods of assessment alternative to statistical analysis (e.g. comparison with laboratory background data).

For larger species (cats, dogs, pigs and primates) having a particular value for certain special investigations, the number of animals should be as large as practicable in order to obtain reproducible results.

In these more expensive animals group numbers lower than 10 are conventionally employed. For primary screening there is no scientific justification for this policy and indeed, because of the lack of background information on such species, even larger group sizes would be desirable.

Equivocal results in initial screening tests should be clarified by further experimentation. At this second stage the investigator may have a particular parameter to investigate and therefore can design the study according to correct statistical procedure. Alternatively, the investigator may adjust the dosage and/or dosing period to achieve a more pronounced and recognizable response. It is at this stage of testing that investigators may be able to use the smaller numbers of larger species.

5. Statistical analysis

For teratology studies the basic aim is to determine whether or not a biologically important event has occurred and whether or not it is related to treatment. Undoubtedly the best way to achieve this aim is to design and conduct the study in such a way that the result is self-evident. As experiments are rarely so perfect an investigator may have to resort to additional

aids to assess the meaning of results and plan the next step in the sequence of investigation.

In deciding which method of statistical analysis is the most appropriate the investigator must determine

- the truly independent variables
- their type of distribution.

In teratology studies, doses are usually given to the parent animal and not directly into each individual embryo or foetus; on this basis the litter may represent the unit. The alternative possibility is the analysis based on the individual foetuses, common examples being:

a) Chi² test or Fisher's Exact test on group totals, proportions of live and dead implants, and of abnormal and normal foetuses

b) Analysis of implantation ratios by t-test

c) Analysis of mean pup weight by t- or u-test.

Regarding distributions, litter parameters are usually represented by small discrete variables with only corpora lutea counts and implantation rates approaching the so-called normal (Gaussian) distribution; the distribution of variable young is skewed as well as being composed of discrete intervals whilst the incidence of dead young (resorptions) and abnormal young tends to follow a Poisson distribution. This peculiarity of litter parameters effectively devalues the reliability of statistical analysis such as the Student's 't' test and variance analysis based on the normal curve for continuous variables.

In general terms investigators will find that in most cases, but not all, non-parametric methods will provide the simplest and most effective methods of analysing the results of teratology studies. For example, for all-or-none litter responses such as non-pregnancy, total resorption and/or abortion, Fisher's exact test (or tables of hypergeometric progression) is one of the more useful methods of assessment; when control values are nil or very low a one-tailed criterion should be employed. As an alternative the Chi² test may be used if there are a sufficient number of litters (i.e. not less than 5 in each compartment of a 2×2 contingency table).

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:

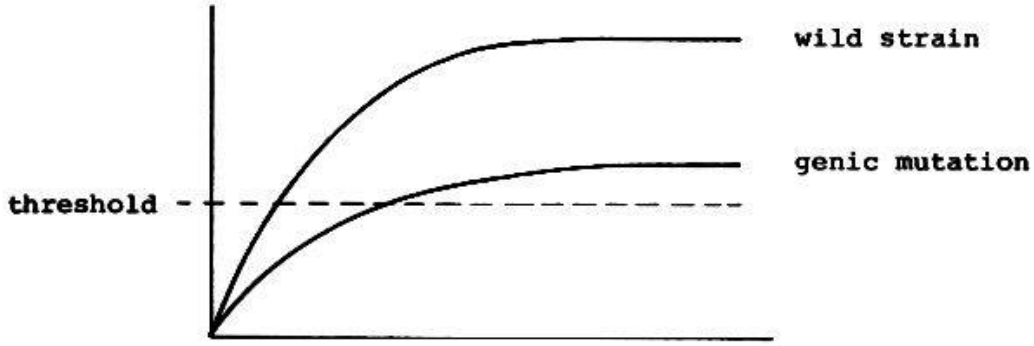
- The susceptibility is not a general one: the gene action is organ-specific. For instance, 6-amino nicotinamide induces a high percentage of vertebral anomalies in a mouse strain but practically no cleft palate.
- The tendency to malformation of one organ may depend on several genes, each being affected 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-

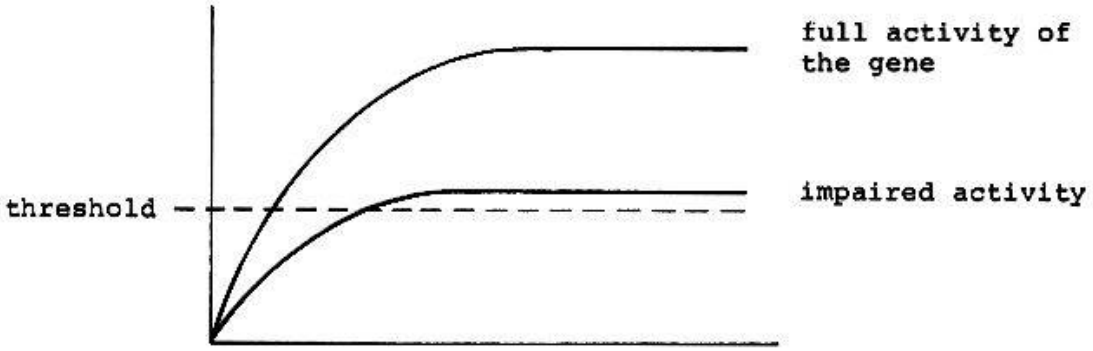
A = Genetic activity controlling the development of an organ

1. Mendelian susceptibility



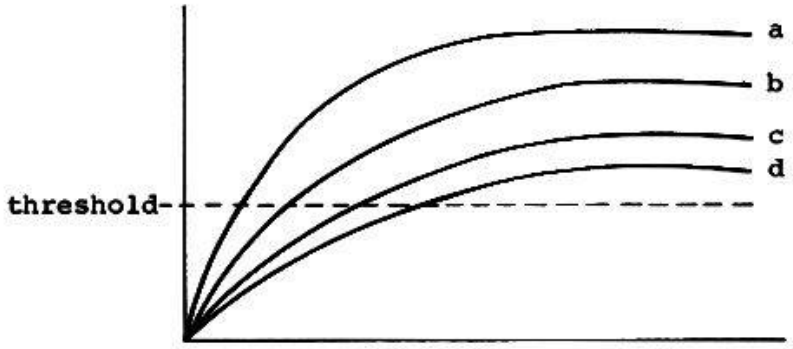
1- The new gene resulting from the mutation has a lower activity. It is close to the threshold. A "weak" teratogenic agent can bring it below the threshold, resulting in a malformation.

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

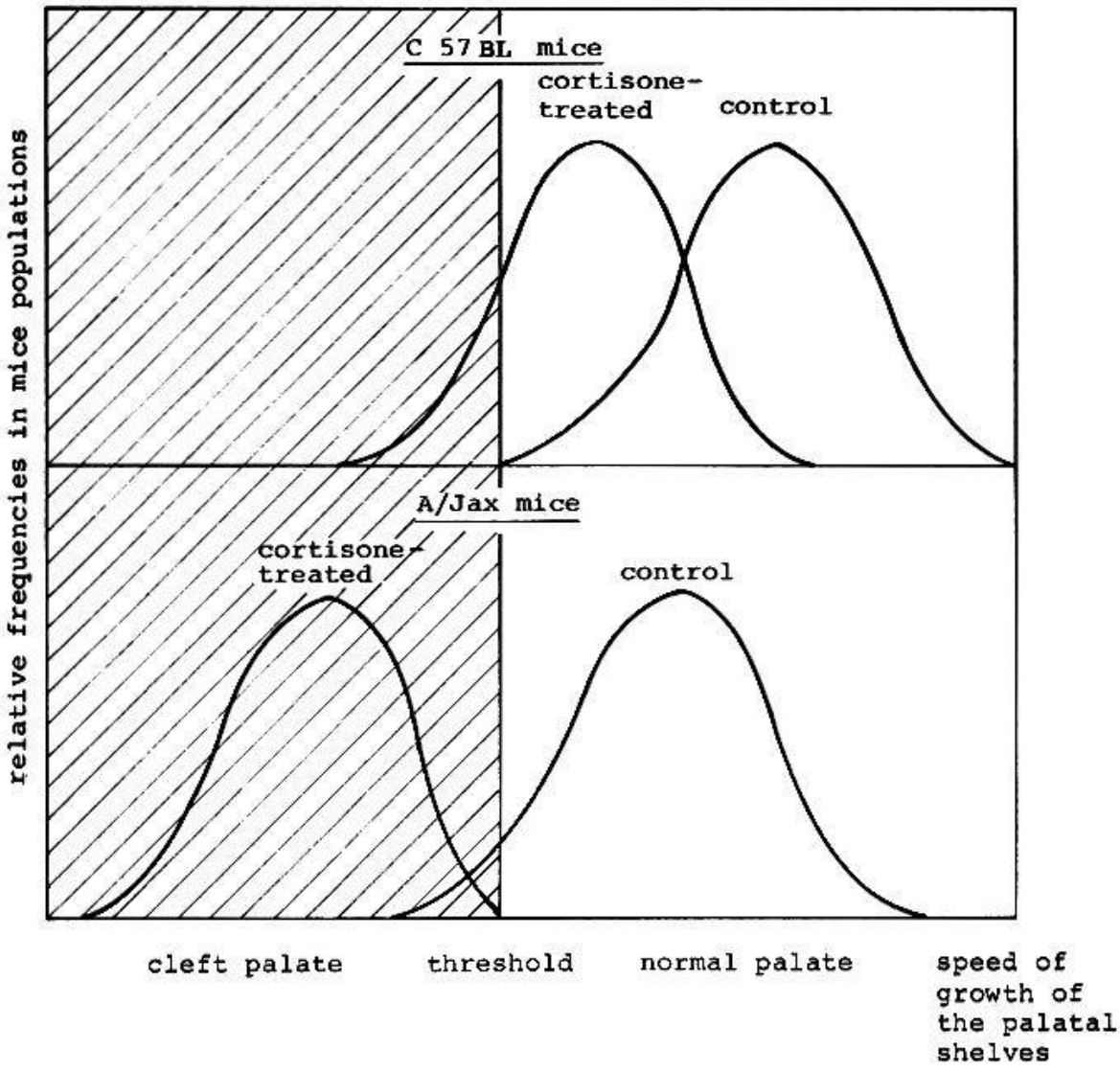


Fig. 8. The postulated mechanism of the differential susceptibility of two inbred strains of mice to cortisone concerning the induction of cleft palates.

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.

VI. CONCLUSIONS

Among the various adverse effects of drugs and other chemical agents, their potential action during intra-uterine life is of particular concern because of its irreversible nature.

In order to present a critical evaluation of the present methods of assessing teratogenicity, it was necessary to make as complete an analysis as possible of the various factors involved in the production of congenital malformations.

Although it was recognized that certain agents might act directly on the genetic material by inducing mutations which lead to developmental defects, the group did not discuss mutagenicity testing because this particular problem has recently been treated by a panel of the World Health Organization.

Therefore particular attention was devoted to the aetiology of congenital malformations with special reference to the role of genetic factors, the importance of the physiology of prenatal development, and placental function. This required an extensive review of the available methods for detecting teratogenic potential of drugs and other chemical agents to which the maternal organism may be exposed. Therefore, the conditions involved in teratogenic reactions, the selection of animal species, the choice of doses, the number of animals, and the predictive value of experimental data as well as the eventual priorities in teratogenic drug testing, were considered.

Malformations may be induced in man by external agents (especially by drugs) and they must be viewed against the background of spontaneous malformations. An appreciable number of them are considered to be of genetic origin, i.e. due to point mutations or chromosomal aberrations.

The nature and origin of spontaneous malformations occurring in man is of prime importance in formulating a general concept of methods that may be considered adequate to analyse the possible factors in the laboratory animal.

Chromosomal changes may be diagnosed by karyotypic studies. They represent about $^1/_{10}$ of the total number of human malformations. Point mutations are recognized by their hereditary transmission. Their incidence is estimated at less than $^1/_{10}$ of the total malformation rate. The majority of the malformations are considered to be of multifactorial origin to which exogenous factors may contribute, although at an unknown rate.

Two types of method are used in the study of the aetiology of congenital malformations: prospective or retrospective epidemiological studies, and animal experiments. Although epidemiological investigations have yielded a number of valuable results, the interpretation of the data is beset with difficulties because of the large number of potentially causal factors that might intervene in human pregnancy. By contrast, experimental investigations are performed on laboratory animals under strictly controlled conditions.

The basic principles of testing for teratogenicity are similar to those underlying the detection of general toxicity except that the action of an injurious agent on the embryo is more complex than its action on the adult. In terato-

genesis, one is dealing with two interdependent biological systems, the pregnant female and the embryo; the specific reactions of each may be entirely different.

The embryo encounters profoundly changing conditions as functions are assumed while the organs are developing. The initial stages of the growing conceptus are in fact governed by effects at the cellular level. Thereafter, morphogenetic movements determined by sequential induction processes mark the modelling of the early embryo. In this most critical period the specific organ functions have not yet developed and detoxification processes such as are present in the adult organism are not available. This is the phase of greatest sensitivity to teratogens. Specific enzyme functions characteristic of the future organs begin in the anlagen. The general metabolic functions are oriented to anaerobic glycolysis. Later on, when the embryo differentiates into a foetus, most metabolic functions are still taken care of by the maternal organism, the foetal functions being restricted to circulation and renal excretion. Certain organ systems which differentiate at later stages, like the external genitalia, or histogenetic processes which last for the entire prenatal period, like the nervous system, remain vulnerable to factors interfering with their development.

At birth, metabolic functions are switched to catabolism. All the energy previously stored as glycogen and lipid during foetal life is required for thermogenesis, the assumption of respiration, and motility. Early postnatal life is thus partly conditioned by the stage of maturation achieved.

The placental functions are very complex and little understood. Unfortunately this is particularly true of the early trophoblast stages, which coincide with the period of maximum teratogenesis.

Some of the modalities of active or passive transfer of chemical agents via the placenta have been investigated. Although placental transfer is important it is not the essential determinant of embryotoxicity or of teratogenicity. Direct effects on placental function or haemodynamics are possible and these may favour certain malformations, without any transplacental transfer of a chemical being implied.

To produce a congenital malformation, not only has the agent or its metabolites to be present in the appropriate amount, but it also has to act at a very precise moment in the course of morphogenesis. In addition, the embryo must have the suitable genetic susceptibility to react.

To a large extent these conditions can be made to prevail in experiments on laboratory animals. It may be assumed however that they are only exceptionally prevalent during the development of the human foetus. This may be one of the possible reasons why, of a rather large variety of agents that are known to produce congenital malformations in laboratory animals, only a few are suspected of having a teratogenic effect in humans.

Through analysis of the data available at present, an attempt was made to establish a generally acceptable methodology. The agent is administered by the route that is most relevant to human intake.

Several, usually three, dose levels are employed and for each dose 10 to 20 pregnant animals are considered sufficient to give interpretable results. The animals are treated during the entire period of organogenesis or for a limited amount of time when only a sequential method is used.

Special problems are raised by certain chemicals, such as antibiotics capable of altering the intestinal flora, compounds with enzymatic activity, psychotropic drugs which are lactogenic hormone releasers, and steroid hormones. In these cases, primary and secondary effects have to be distinguished.

One of the main difficulties in extrapolating experimental results is due to the fact that the various animal species, or strains of the same species, or even individuals, may vary in their reaction to an injurious agent. To some extent, these difficulties can be overcome by using several species and a large enough number of test animals to produce statistically valid data with respect to the parameter(s) under study.

According to present knowledge, rodents and/or lagomorphs can be expected to yield results that are of relevance to man. The predictive value of studies in these species seems to be at least as great as that of experiments in other species, including non-human primates.

For routine teratogenicity screening at least two species, a rodent species (the rat or the mouse) and a lagomorph (the rabbit), should be used.

The use of more than one species seems necessary because many known teratogens produce a positive effect in only one species. Difficulty in interpretation may emerge, however, when a potential drug or other new chemical substance produces a teratogenic effect in this way. Unless it is possible to explain the factor(s) implicated in this effect and it can be confidently stated that it has no bearing on the situation of the human, possible relevance for man must be assumed.

Until the basic mechanisms of teratogenicity are better understood, it will be impossible to design more relevant testing procedures. Neither the chemical structure nor the biological activities of a compound give a valid indication of its teratogenic potential.

The predictive value with regard to man of results obtained by teratogenic testing is not known at present. In spite of this, there is no alternative way of obtaining some guidance when a possible hazard is to be assessed.

The evaluation of a possible teratogenic risk should be made by taking into consideration all the circumstances under which the results were obtained; e.g. frequency of the anomalies, dose-response relationship, number of species in which anomalies occur, and similarities with reference compounds.

Provided teratogenicity experiments are performed by an experienced investigator under standard laboratory conditions and on a sufficiently large scale, the results may be considered to give a valid and relevant indication of the possibility of interference with important developmental processes. The present testing methods therefore appear to be satisfactory as far as safety evaluation is concerned. It must be borne in mind, however, that

animals may have a specific teratogenic susceptibility and may yield "false positive" results. Therefore the interpretation of positive laboratory data can only be attempted by taking all the known aspects of the biological activity of a compound into consideration.

From the experimental data gained in recent years it is concluded that suspected noxious actions of drugs on the embryo were detected by proper experimentation.

The characteristics of a compound must be taken into account when discussing priorities of testing, e.g. chemical structure and intended use.

In general, animal tests for teratogenicity should be performed in support of clinical trials of a new drug.

Tests should also be extended to known drugs in widespread use. Compounds of that sort offer the advantage of the large experience gained in a large human population for a comparatively long period of time. Therefore it is of considerable scientific interest to evaluate such compounds in teratogenicity studies on animals. Such information may form the basis for future attempts to estimate the predictive value with regard to humans of animal experiments in this field.

The prospects of identifying teratogenic agents seem favourable and as more experience is gained by animal experiments and human epidemiological studies, preventive measures may become a possibility.

Since, among many predisposing factors, the genetic component may be an important determinant in experimental teratogenicity experiments using particularly susceptible species or strains of laboratory animals, it may be useful to elucidate the importance of different genetic mechanisms in the development of congenital malformations.

The development of these different lines of investigation may lead in the future to a better insight into teratological mechanisms and consequently to valuable improvements in teratogenic drug testing.

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