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The application of the results of experimental research work on animals to man

By J. W. Millen, Cambridge

Recent events have created a great upsurge of interest in the problem of congenital malformations in man, and clinicians have naturally turned to the basic medical sciences for information, from animal experiments, about the possible causes. Unfortunately, there has sometimes been a regrettable, though understandable, tendency to an uncritical assumption that the results of experiments in animals are equally valid for man. It may be well, therefore, to consider to what extent animal experimentation can aid in a better understanding of the causes of human malformations and to examine the factors which must be taken into account in the extrapolation of the results of such experiments to man.

The earliest teratogenic experiments were carried out by *E. G. Saint-Hilaire* (1822) and he was also responsible for coining the word teratology for the science concerned with the study of malformations. *Saint-Hilaire* discovered that by varnishing the shell of a hen's egg, and thus cutting off the oxygen supply to the developing embryo, he could produce anencephaly and spina bifida. Since this discovery was made, over 100 years ago, investigators have studied extensively the production of embryonic defects in animals by a great variety of experimental methods, but only in the last 30 years or so have these experiments been applied to mammals. Until the publication of *Hale*'s (1933) researches on the effects of depriving sows of vitamin A during pregnancy, it was generally held that the mammalian embryo was a perfect parasite protected by the mother from deleterious influences except those due to defective genes.

From the standpoint of experimental teratology animals may be divided broadly into three categories:

1. Birds. The embryo has within the egg an adequate food supply for its nutrition and is dependent on the external environment only for

oxygen and heat. It is protected from injurious influences by a hard outer shell.

2. Amphibia and most fishes. The embryo is bathed in a fluid medium from which it is not insulated and upon which it is dependent not only for oxygen, but also for water and inorganic elements.

3. Placental mammals. The embryo develops within the maternal organism and is wholly dependent upon it for all its nutritive requirements after implantation. It is, however, separated from the internal milieu of its mother by a placenta and is shielded from changes in the external environment by the maternal organism. This protection is not complete for as *Needham* (1931) has pointed out there appears to be a point at which no further calls can be made upon the maternal tissues and the foetus has to bear the brunt of the deficiency. Into this category fall all the mammals, except the ornithorhynchus and echidna, and it includes man.

Much of the early experimental work on teratology was carried out on avian and amphibian eggs. Scientists discovered that malformations could be produced by mechanical stimuli, by physical agents, and by the introduction of chemical substances into the environment of the developing embryo. These researches have added greatly to our knowledge of the effects of alterations in the environment upon the development of the embryo, and still provide fruitful fields for further exploration. The conditions under which these embryos develop are, nevertheless, very different from those of development in placental animals where teratogenic influences must operate not directly upon the embryo (with the possible exception of irradiation), but through the medium of the maternal organism. This interposition of the maternal organism allows of the modification of the effects of potential teratogens either to diminish or perhaps, in some cases, to augment their effects. On the other hand, in non-placental animals the teratogen acts directly upon the developing embryo and its effects are not subject to any modifying influences exerted by the mother.

Many problems are posed when one attempts to extrapolate from the results of animal experiments to man. In animal experiments it is generally possible to control quite strictly the conditions of the experiment so that only the factor under investigation is varied, whereas in a human population the interpretation of the effects of a suspected teratogenic agent must be made against an infinitely variable background of race, environment, social status, dietary habits, and local conditions peculiar to the woman herself, such as infection, hormonal state, multiple pregnancy and, perhaps, psychological disturbances

(Table 1). Nor are the components of this background clearly separable, but are so interwoven with one another as to provide a veritable kaleidoscope of constantly changing conditions.

It is to some of the elements in this background and to the knowledge, derived from experimental work, concerning their effects on development that I should like to direct your attention.

Table 1
Factors which may influence teratogenic activity in man

Race
Environment
Social status
Dietary habits
Local conditions
a) Infection
b) Hormonal state
c) Multiple pregnancy
d) Psychological disturbances (?)

Genetic factors

The question of the genetic causes of malformations is outside the scope of this paper, and I will content myself with reminding you of *Fraser*'s (1959) conclusion at a conference on congenital malformations that a minority of congenital malformations have a major genetic cause.

Nevertheless, genetic factors are undoubtedly of importance in relation to malformations whose appearance is not, at least directly, due to defective genes.

Species differences in the effects of teratogens are well-known and I shall only instance two examples. Vitamin A deficiency during pregnancy was the earliest environmental teratogenic agent, other than X-irradiation, to be investigated experimentally (*Hale* 1933). *Hale* found anophthalmia, skeletal malformations and renal anomalies in the young born to sows fed on a vitamin A-deficient diet for about four months before mating. Subsequent investigators confirmed Hale's findings and also observed cardiovascular anomalies and diaphragmatic herniae (*Wilson* and *Warkany* 1949, *Andersen* 1941). Most of these experiments were carried out on rats and abnormalities of the central nervous system were rarely noted. However, when, at Cambridge, female rabbits were subjected to a vitamin A deficiency before mating, it was discovered that many of the young were hydrocephalic (*Millen*, *Woollam* and *Lamming* 1953). Furthermore, there was a direct relationship between the incidence of hydrocephalic young and the duration of the vitamin

deficiency (Fig. 1) (*Millen* and *Woollam* 1956). But the only account of hydrocephalus due to vitamin A-deficiency in another species is that of *Rokkones* (1955) who obtained hydrocephalic young in the second and subsequent litters of female rats maintained from weaning on a diet low in vitamin A. It appears reasonable to conclude that rabbits are more susceptible than other species to the production of hydrocephalus due to vitamin A-deficiency.

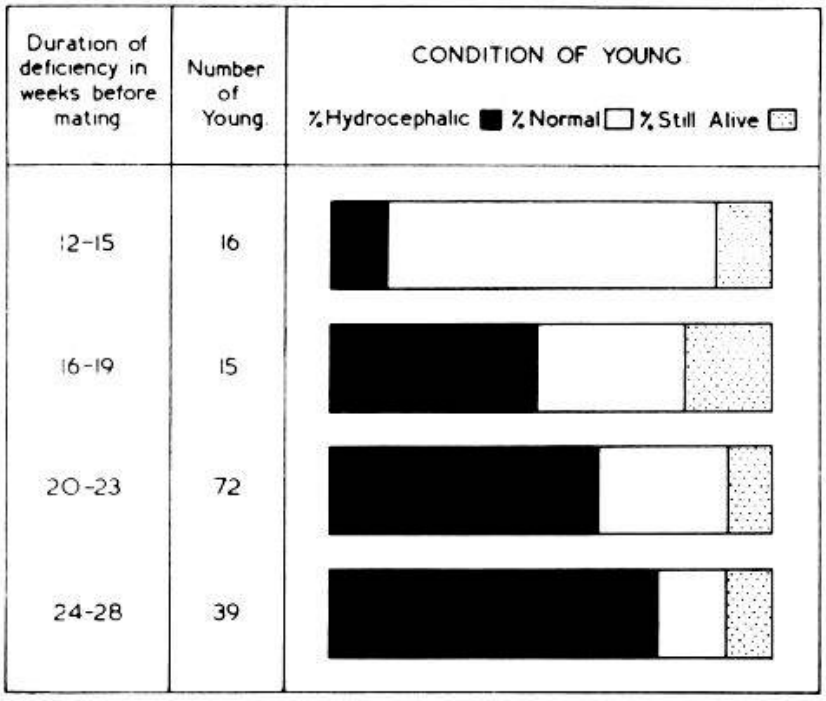

Fig. 1. Diagram illustrating the effect of the duration of vitamin A deficiency in rabbits on the incidence of hydrocephalus in their young. (From *Millen* and *Woollam:* J. Neurol. Psychiat. **19,** 17 [1956]).

The other example is fresh in the minds of all of us. When it was discovered that thalidomide was responsible for the sudden increase in the incidence of babies born with severe limb malformations, research workers everywhere immediately examined the effects of the drug on pregnant laboratory animals. Rather to the surprise of most investigators, it proved extremely difficult to reproduce the malformations found in humans, even when proportionately very much larger doses were used. Many workers failed to obtain any malformations, and apparently only one strain of rabbits, the New Zealand White, shows any marked susceptibility (*Somers* 1961). Even in this strain, the severity of the malformations produced by thalidomide appears to be considerably less than in human babies.

In addition to the species differences in the effect of teratogenic agents, there are, even within a single species, differences between different

strains. *Fraser* and his associates (1954), in a well-conceived series of experiments, demonstrated that in one strain of mice, maternal treatment with 2.5 mg. cortisone daily for four days during pregnancy produced almost a 100% incidence of cleft palate in the young, whereas in another strain the same dose given for the same period resulted in the appearance of cleft palate in only about 20% of the offspring.

In the human population, racial differences are apparent in the incidences of various types of malformation. A comparison was made by *McKeown* and *Record* (1960) of the incidences of different malformations in Sweden, Japan and Birmingham, England. They found (Table 2) that the incidence of malformations of the central nervous system—anencephaly, spina bifida and hydrocephalus—was much higher in Birmingham than in Sweden (*Böök* 1951) or in Japan (*Neel* 1958). On the other hand, the incidence of cleft lip and palate was higher in Japan than in either Sweden or Birmingham. Revised estimates for Japan (at nine months) and for Birmingham (at five years) reflected the same differences. Dislocation of the hip, which was relatively rare in Birmingham, was ten times as common in Japan. While some of these differences may be affected by environmental factors it seems probable that they are determined largely by racial heredity.

Penrose (1957) made an intersting study of the geographical distribution of a single malformation, anencephaly (Fig. 2). He found that

Table 2
Incidence of malformations (per 1000 total births)
(From *McKeown* and *Record*, 1960, in Ciba Foundation Symposium on Congenital Malformations)

Type of malformation	Estimates soon after birth			Revised estimates	
	Swedish data (44,109)	Japanese data (64,570)	Birmingham (56,760)	Japanese data (9 mths.)	Birmingham (5 years)
Anencephalus	0.54	0.63	1.96	0.63	1.96
Spina bifida, etc.	1.09–1.45	0.26	2.80	0.32	3.00
Hydrocephalus	1.00	0.32	1.76	0.50	2.57
Mongolism	0.48	0.09	1.11	0.87	1.69
Cardiac malformations	0.79	4.21	2.11	6.97	4.18
Cleft lip and/or palate	1.75	2.78	1.76	2.96	1.94
Talipes	2.79	1.10	3.95	1.40	4.44
Dislocation of hip	0.00	0.31	0.02	7.13	0.67
All malformed individuals	11.18	12.22	17.30	24.54	23.08
Individuals excluded	2.27	1.16	0.0	4.81	0.0

Numbers in brackets are the populations (total births) in which the malformations were identified.

in Europe the incidence of the malformation was very high in Ireland (0.671% in Belfast) and in western England, but lower in the remainder of England, and declining on the continent of Europe to 0.012% in Lyons. Again, one cannot exclude environmental factors, but it seems likely that some of the difference is to be accounted for on a genetic basis.

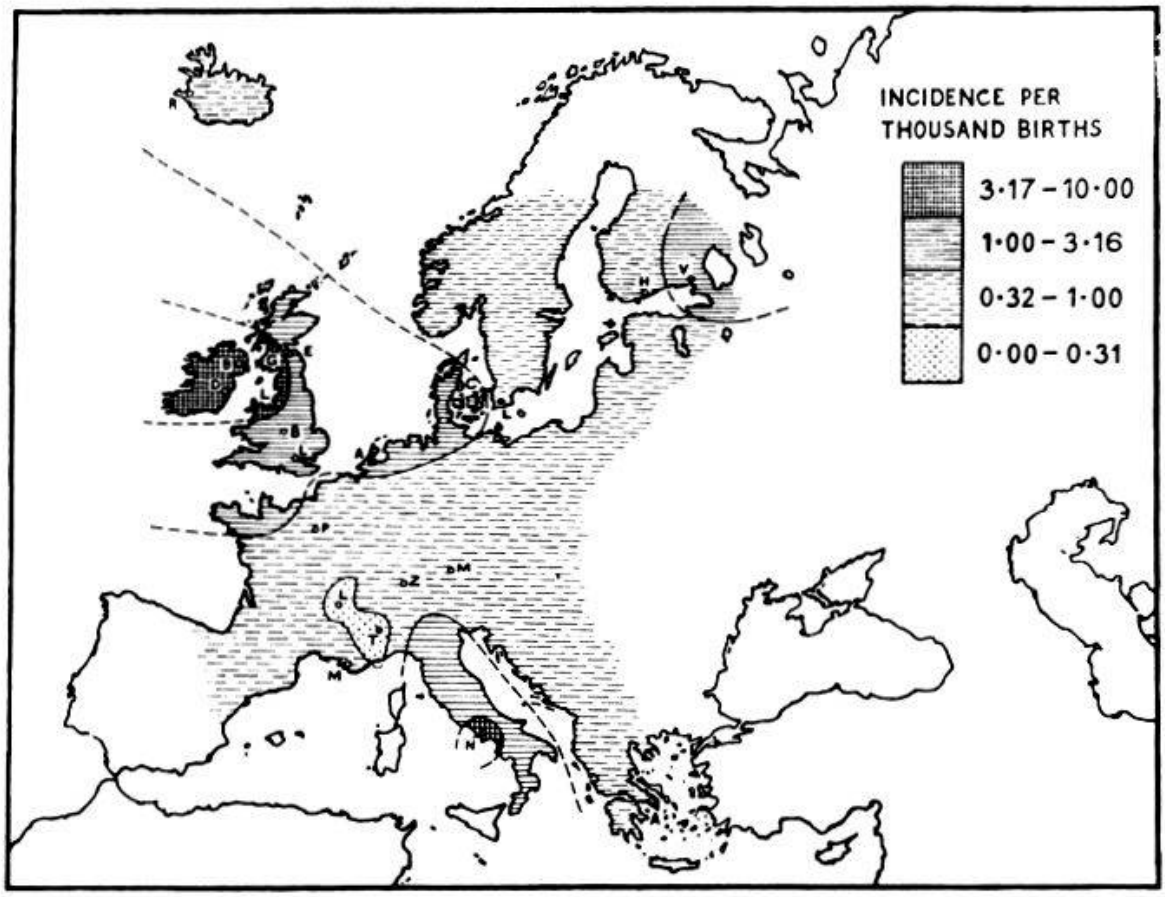

Fig. 2. Distribution of anencephaly in Europe based upon births in hospital. (From Fig. 1, *Penrose:* J. ment. Def. Res. **1**, 4 [1957]).

Another aspect of the influence of genetic factors was investigated by *McLaren* and *Michie* (1961) who showed, by the use of an ingenious experimental technique, that the expression of a genetically determined malformation depends not only upon the foetal genotype, but also upon the effect of the maternal genotype on the uterine environment. They found by reciprocal crosses between two strains of mice, C_3H and $C_{57}BL$, which have predominantly 5 and 6 lumbar vertebrae respectively, that the first generation offspring tend to resemble the maternal strain in the number of lumbar vertebrae rather than the paternal strain. However, when they transferred embryos to foster mothers of the paternal strain, the young now resembled their foster mothers, rather than their true mothers, in the numbers of lumbar vetebrae (Fig. 3).

Diet

Under standardized laboratory conditions, it is possible to control the diet of experimental animals within narrow limits. The production of maternal dietary variations and the study of the effect of these variations upon the development of the embryo can therefore be investigated with considerable facility.

A great deal of the experimental work on the problem of mammalian teratology has been concerned with effects of a deficiency of different vitamins in the diet upon embryonic development. By this means it has

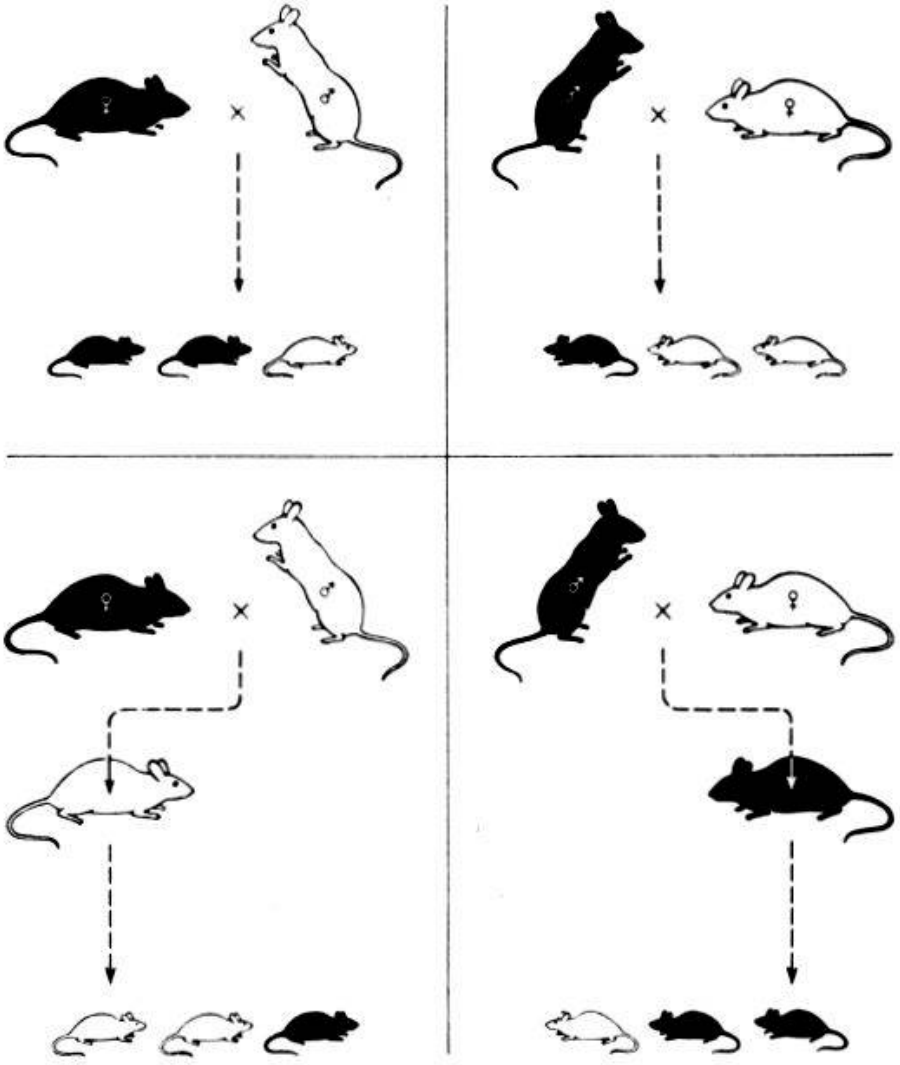

Fig. 3. Diagram to illustrate the experiment of *McLaren* and *Michie*. Mice with 5 and 6 lumbar vertebrae are represented as black and white respectively. The 2 upper pictures represent reciprocal crosses between the C_3H and $C_{57}BL$ strains of mice, which have predominantly 5 and 6 lumbar vertebrae respectively. The F_1 progeny tend to resemble the maternal strain in vertebral type. In the 2 lower pictures, F_1 hybrid embryos are transferred to uterine foster-mothers of the paternal strain, and when born are found to resemble their foster-mothers rather than their true mothers in vertebral type. (From Fig. 4, *McLaren*, 1961, in: First International Conference on Congenital Malformations.)

been shown that in addition to vitamin A-deficiency, to which reference has already been made, deficiencies in the maternal diet of some of the B group of vitamins—thiamine (B_1), riboflavin (B_2), nicotinamide, pantothenic acid, folic acid and vitamin B_{12}—and of vitamin E provoke the appearance of malformations. Dietary deficiencies in these vitamins can result in malformations of the central nervous system, the cardiovascular system, the genito-urinary system and the skeletal system. Although the syndromes of malformations produced by deficiencies in individual vitamins show some variations, nevertheless the wide range of deformities that can be caused by a deficiency of any one vitamin is very striking. Such a correspondence suggests that the effect of vitamin deficiencies is to produce not a purely local effect, but a profound metabolic disturbance whose effects are to some extent modulated by local circumstances within the embryo. This is not perhaps surprising when one takes into account the importance of the B vitamins as co-enzymes in protein and carbohydrate metabolism. The reason for the teratogenic activity of a vitamin A-deficiency is more puzzling, probably because, except in the synthesis of rhodopsin, little is known of its role in metabolism. Vitamin A is also unique among the vitamins in that the feeding of excessive amounts of the vitamin during pregnancy is also highly teratogenic and produces a broad spectrum of malformations.

Apart from the effect of specific deficiencies of nutrients upon normal development, malformations can be produced in experimental animals by fasting. In mice, which appear to be particularly susceptible, *Kalter* (1954) found a moderate incidence of cleft palate in the offspring of young female mice starved for 72 hours during pregnancy. In another strain of mice, *Runner* and *Miller* (1956) found that 24 hours fasting on the 9th day of pregnancy produced abnormalities in 28% of the young. These effects were completely abolished by feeding glucose during the period of fasting.

A further refinement in experiments on the effects of fasting was introduced by the concurrent administration of hormones. *Smithberg* and his collaborators (1956) found in mice that a period of only 6 hours fasting was sufficient to produce malformations in 63% of the offspring when the fasting was associated with the administration of 0.1 units of protamine zinc insulin. In another series of experiments, *Kalter* (1960) studied the effect of combining a restricted diet with small doses of cortisone, which is known to induce cleft palate and lip in mice (*Baxter* and *Fraser* 1950). The results of these experiments showed that whereas on a restricted diet alone the incidence of cleft palate was 5.6%, when, in addition, 1.0 mg of cortisone was administered for four days during pregnancy, the inci-

dence of cleft palate in the young rose to 50.7%. In other experiments, *Kalter* (1956) observed that the incidence of cleft palate produced by a standard dose of cortisone decreased significantly with increasing maternal weight. He suggested that greater fat resources may exert a protective effect in the larger animals.

When one comes to consider the application of observations upon the effect of dietary variations in experimental animals to the human population, the problem becomes at once immensely complicated. The dietary habits of the human population are much less controllable than those of a group of laboratory animals, and are affected by a wide variety of factors such as climate, social habits, economic circumstances, religious mores, seasonal variations in the availability of certain foods. war-time restrictions and personal idiosyncrasy (Table 3).

Table 3
Some factors which may influence dietary habits

Climate
Social habits
Economic circumstances
Religious mores
Seasonal variations in availability of foods
War-time restrictions
Personal idiosyncrasy

Dietary deficiencies in vitamins sufficient to produce clinical manifestations appear to be relatively rare, and in none of the studies carried out on women in pregnancy has there been clear-cut evidence that the occurrence of malformations could be attributed to deficiency in any one vitamin. It may be desirable, however, to echo the warning given by *Spies* (1958) of the importance of subclinical deficiencies in vitamins which do not manifest themselves by the classical syndromes. Nor should one forget that in experimental animals it is the moderate deficiencies, which do not seriously affect the mother or interrupt pregnancy, that are of the greatest importance in relation to the occurrence of malformations.

Animal experiments on the relationship between the restriction of food intake and the occurrence of malformations are supported by the results of a number of studies made on human populations.

An increase in the malformation rate during and immediately following the Second World War was observed among women in Leipzig by *Aresin* and *Somner* (1950), and a slight increase in the number of congenital deformities was reported among babies born in Holland following a period of severe hunger in 1944 and 1945 (*Smith* 1947). In a survey

carried out in Boston, *Burke* and her co-workers (1943) found that most babies with major congenital defects were born to mothers receiving the poorest diets. Again, *Baird* (1945) reported that in Aberdeen the incidence of fatal malformations was twice as high in babies born to women in the lower economic and social classes as in babies born to women in the upper economic and social classes. He attributed the difference to poor health and nutrition in the former group.

These and similar reports would appear to confirm the observation that malnutrition in humans has the same teratogenic effects as in animals, were it not for the fact that other observers carrying out comparable investigations have denied any evidence of a relationship between nutritional factors and the incidence of human malformations (*Murphy* 1947; *Ferrario* and *Fortina* 1950; *Edwards* 1958).

Metabolism

In attempting to apply the results of animal experiments to man it is important to remember the differences in metabolism, not only between man and other animals, but also between different species of experimental animals.

For example, the metabolic requirements of different animals for exogenous sources of the vitamins are not identical. Vitamin B_1, thiamine, which acts as a catalyst in carbohydrate metabolism, is formed in the intestine when fermentation occurs, as in ruminants. Or again, folic acid, another B vitamin, which is essential in the oxidation of aromatic amino-acids, is continuously synthesized by bacteria in the intestine of the rat. Rats do not therefore require an extrinsic source of the vitamin, and in experimental work employing folic acid deficiency it is necessary to suppress synthesis by the administration of succinyl sulphathiazole. The requirements for folic acid in man are not exactly known despite its importance in the treatment of anaemia. It is, however, of particular interest that aminopterin, a folic acid antagonist, is one of the few substances which have been shown to be teratogenic both in man and in experimental animals.

Nor should it be forgotten that the basal metabolic rate varies considerably in different animals (Fig. 4). The laboratory animals most commonly employed experimentally, namely the mouse, the rat and the rabbit, all have much higher basal metabolic rates than man. It is necessary to remember these differences when considering the results of experiments on animals in which hormones that alter the metabolic rate are employed as teratogens, or to modify the action of known teratogenic

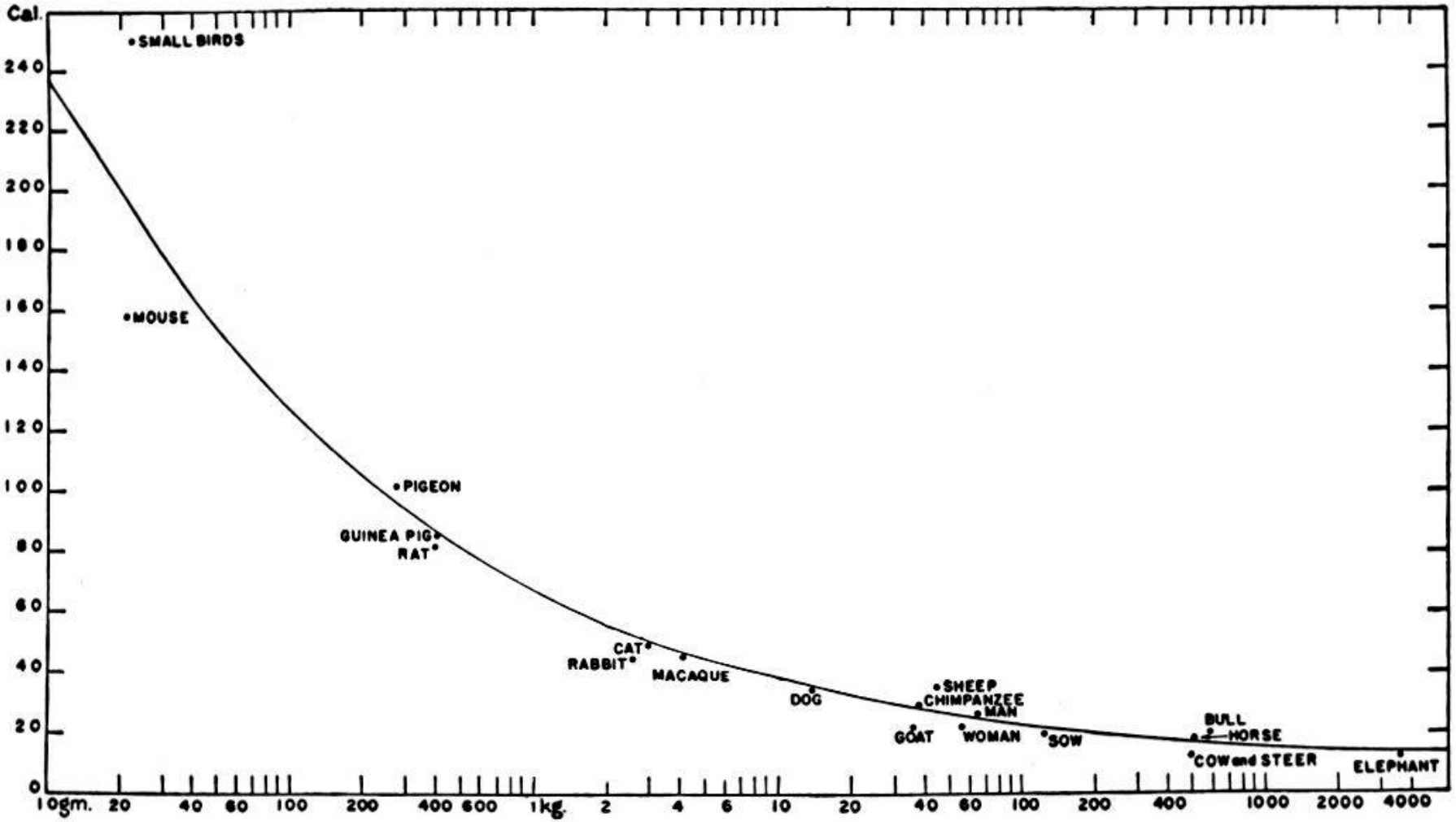

Fig. 4. Relationship between average basal metabolic rate per kg per day of different species (vertical axis) and average body weight (abscissa). The weight range is from 20 g to 4000 kg. Semilogarithmic chart. (After *Benedict* in: Carnegie Instn. Wash. Publ. 503 [1938].)

substances. For instance, insulin has been used as a teratogenic agent in rabbits by *Chomette* (1955), but has been found by *Millen* and *Woollam* (1958) to diminish the teratogenic activity of vitamin A in rats. On the other hand, in mice an alloxan-induced diabetes has been found by *Ross* and *Spector* (1952) to be teratogenic. In this regard, one may recall that *Hoet* and his collaborators (1960) have found that congenital malformations in babies are frequently associated with pre-diabetes in pregnancy.

Another example of the effect of hormones which alter metabolic activity is provided by the results of experimental work in which thyroxin or an antagonist, methylthiouracil, have been employed. In the experiments of *Giroud* and *Martinet* (1954), congenital cataract was found in the offspring of female rats given 0.25–0.50 mg of thyroxin daily by mouth before and during pregnancy. These investigators also noted an interesting strain difference after oral treatment from the 9th to 20th days of gestation. Wistar rats were found to be much more susceptible, 20% of the young being affected, than Norwegian piebald rats in which only 1.7% of the young had abnormal eyes. In another series of experiments (*Millen* and *Woollam* 1959; *Woollam* and *Millen* 1958), methylthiouracil was found to increase the incidence of cranial malformations and clefts of the palate in the offspring of female rats treated with large doses of vitamin A, whereas the administration of thyroxin appeared to exert a protective effect.

The importance of maternal thyroid activity in the human population is well illustrated by the association of cretinism with geographical regions where an insufficiency of iodine is present in the water, and it has been claimed, although not universally accepted, that there is in general a higher incidence of malformations in areas where cretinism is endemic (*Eggenberger* 1928).

Stress

It is an ancient belief that maternal impressions and emotions, which we would to-day call stress, play an important role in the causation of congenital malformations. *Selye* (1950), who made an exhaustive study of the pathological effects of stress, believed that stress during pregnancy, mediated through the hypophysis—adrenocortical mechanism, could cause malformations in the baby. In a retrospective human study, *Stott* (1957) found evidence which supported Selye's view. *Stott* observed that stressful occurrences were commoner in pregnancies resulting in the birth of a malformed child than in normal pregnancies. The malformations considered by *Stott* were of many varieties, but *Strean* and *Peer*

(1956) reached similar conclusions from an investigation of the case histories of women who had borne children with cleft palates.

If stress is equated with an abnormal production of corticosteroids by the mother, then some support is afforded by the work of *Fraser* and his collaborators (1954) who have used cortisone as a teratogen for the production of cleft palate in mice. In the rat, however, *Woollam* and *Millen* (1957) were unable to produce cleft palate by cortisone alone, even when large quantities were given. On the other hand, they found that cortisone produced a marked increase in the number of young with cleft palates when administered concurrently with excess vitamin A.

A note of caution in the too-ready acceptance of the equation of stress with an overproduction of cortisone is sounded by experiments conducted by *Warkany* and *Kalter* (1962). These investigators subjected pregnant mice, of a strain known to be extremely susceptible to the teratogenic influence of cortisone, to prolonged audiogenic stimulation. They found no increase in the incidence of cleft palate in the offspring of these females despite the marked excitement produced by the stimulation.

Placentation

In comparing the teratogenic effects in different species of mammals, the interposition of a placental barrier between the mother and foetus must constantly be borne in mind. Despite the valuable information which has been derived from experiments on avian eggs and from amphibia, the conditions of development in these animals differ from those pertaining in placental mammals, where not only all nutrient and waste products have to cross the placenta, but the placenta itself has endocrine functions which may affect embryonic development.

Even in placental animals there are morphological differences in the structure of the placenta in various species, and these differences are reflected in the permeability of the placenta to chemical substances. On morphological features, placentae can be divided into four main categories: epithelio-chorial as in the horse, pig, cow, sheep; endotheliochorial, in the dog and cat; haemo-chorial, in man and other primates; and haemo-endothelial, in the rabbit and rat. Gross permeability differences exist between these four principal types of placenta: for example, antibodies, which in the haemo-chorial or haemo-endothelial types pass freely from mother to foetus, do not cross placentae of the epitheliochorial type. These permeability differences depend, to some extent at least, upon the structure of the "placental membrane", that is the tissues

Table 4

The tissues making up the separation membrane in the four principal types of placentation. (From *Hamilton*, *Boyd* and *Mossman*, 1962, Human Embryology)

Type of placenta	Epithelio-chorial	Endothelio-chorial	Haemo-chorial	Haemo-endothelial
Maternal tissue:				
endothelium	+	+	−	−
epithelium	+	−	−	−
Foetal tissue:				
chorion	+	+	+	−
endothelium	+	+	+	+
Familar examples	Horse, pig, cattle	Cat, dog	Man, monkey	Rabbit, guinea pig, rat
Main zoological groups	Artiodactyla Perissodactyla Cetacea Manidae Lemuroidea American mole	Carnivora Bradypodidae Tupaiidae European mole	Primates Tarsiidae Sirenia Megachiroptera Most Microchiroptera Hyracoidea Myrmecophagidae Dasypodidae Most Insectivora Lower Rodentia (Sciuridae Myomorpha)	Higher Rodent. (Leporidae Geomoidea Hystricomorpha)

which separate the maternal and foetal blood in the functional parts of the placenta (Table 4).

Furthermore, within the group of animals with haemo-chorial placentae, differences in placental constitution exist and their permeability characteristics are not uniform. The question of placental transmission is much too vast to be considered in this review, but two examples of the kind of variation that may have importance in relation to the interpretation of experimental results will be mentioned by way of illustration. The presence of glycogen in the placenta appears to be concerned with the structure and growth of the placenta as a transfusion agent (*Huggett* and *Hammond* 1958). According to *Szendi* (1936), the highest concentration of glycogen in the human placenta is found at the 20th day after conception, whereas in the rabbit and rat the maximum concentration is reached about two-thirds through pregnancy. These differences may well affect the transmission of potential teratogens from mother to embryo. The second example is the alterations in placental permeability,

which occur with advancing pregnancy (*Flexner* and *Gellhorn* 1942). In some animals this increased permeability may be related to the change in later pregnancy from a haemo-chorial to a haemo-endothelial type of placenta (Fig. 5).

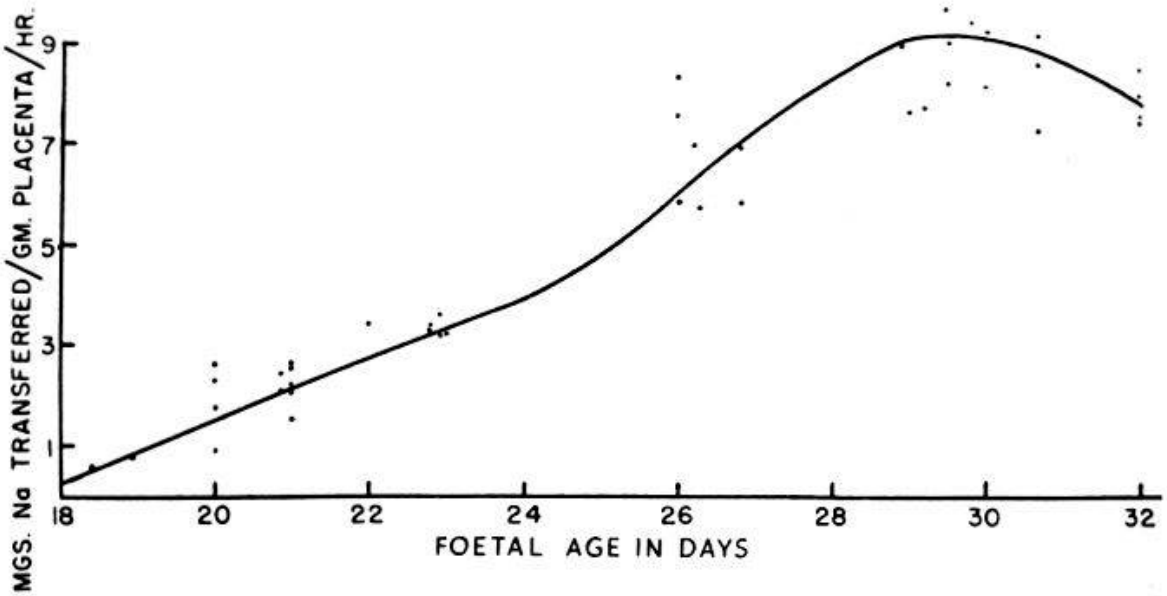

Fig. 5. Graph showing the permeability of the rabbit placenta to sodium at different foetal ages. (From *Flexner* and *Gellhorn:* Amer. J. Obstet. Gynec. **43,** 965 [1942].)

Multiple births

All experimental teratologists have observed that when a teratogenic substance is administered during pregnancy, the degree of injury inflicted on the embryos is not uniform. It is not unusual to find in a single litter that some of the young are dead and in process of resorption, others are malformed in varying degrees, whilst still other embryos appear to have escaped unharmed. Some of this diversity of effect may be due to slight differences in the developmental ages of the embryos or to their position in the uterus, although here the reports of experimental observations are difficult to interpret (*Trasler* 1960; *Woollam* and *Millen* 1961). But the reasons for the apparent immunity of some embryos whilst others are malformed provides a problem which remains for the present unsolved, despite the theories that have been put forward.

In man the frequency of multiple births varies in different countries and between races. The frequency of multiple births in Scotland is said to be one in every seventy-nine births (*Gedda* 1961). About twice as many of these multiple births are due to the fertilization of more than one ovum as are due to the division of a single ovum, that is monozygotic. The greater frequency of malformations found in identical twins than in other individuals is obviously an important question, the answer to

which is bound up with the problem of the diverse effects of teratogenic agents on the individual embryos of polytocous animals.

Other factors

Many other factors may require consideration in the extrapolation of experimental findings to man. Their importance is difficult to assess and space will not allow of any attempt to do more than mention a few of them.

Under laboratory conditions, the season of the year does not appear to play a significant part in experimentally produced malformations, but seasonal fluctuations in the incidences of certain human malformations have been noted (*McKeown* 1961). Again, in the human population some abnormalities are commoner in one of the sexes, but little attention has been paid to this question in teratogenic experiments in animals. To quote another example, pregnant women are liable to infectious illnesses which may or may not be important teratogenically (*Coffey* and *Jessop* 1959; *Wilson* et al. 1959), but experimental animals are either not subject to the same diseases or are intentionally insulated from them. Parental age is yet another factor which has been shown to affect the incidence of malformations, both in man and in other animals.

Conclusions

In conclusion, it seems probable that the ultimate mechanism, or mechanisms, responsible for maldevelopment in animals is some derangement in the metabolism of the embryo at a cellular level. Both carbohydrate and protein metabolism have been implicated, but the precise nature of the disorder remains to be discovered. In as much as the processes of cellular metabolism in all animals have many features in common, the results of experimental researches on lower animals are clearly of great importance for the understanding of the mechanisms underlying maldevelopment in man. Nevertheless, especially in placental mammals there are many variable factors which may affect the outcome of experimental work, and may be of the greatest significance when one attempts to apply the results of these experiments to the interpretation of the causation of human malformations.

Acknowledgments

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Summary

In extrapolating from experimental animals to man it must be borne in mind that many of the factors that can be controlled in animal experiments are infinitely variable in man. The effects of a suspected teratogen must be evaluated against a changing background of race, environment, social status, dietary habits, infection, hormonal state, multiple pregnancy and psychological upsets.

It has been clearly demonstrated by many research workers that, in placental animals, the incidence of malformations, and sometimes the nature of the deformity itself, produced by the use of known teratogenic substances, can be influenced by species and strain differences in the experimental animals employed, by dietary deficiencies, by fasting, by variations in the metabolic requirements of different animals for foodstuffs, and by the injection of certain hormones. Other factors such as placental permeability, the number of young in the litter, and maternal stress during pregnancy, may also be of importance in assessing the effects of teratogens in animal experiments.

Since the ultimate mechanisms responsible for malformations in man and in other animals must be similar, the results of animals experiments are of considerable importance for the evaluation of the causes of human malformations. Due regard must, however, be given to the many factors which may influence the outcome of animal experiments.

Zusammenfassung

Beim Extrapolieren von Versuchstieren auf den Menschen muß man sich bewußt sein, daß manche im Tierexperiment kontrollierbaren Faktoren sich beim Menschen sehr unterschiedlich verhalten. Die Wirkungen eines als teratogen verdächtigten Mittels müssen in Berücksichtigung eines wechselnden Hintergrundes von Rasse, Umwelt, sozialer Stellung, Ernährungsgewohnheiten, Infektionen, hormonalem Zustand, multiplen Schwangerschaften und psychischen Störungen gewertet werden.

Es wurde von vielen Forschern eindeutig bewiesen, daß bei Placentaliern das Vorkommen von Mißbildungen und manchmal die Natur der durch teratogene Substanzen entstandenen Mißbildung selbst, durch Art und Stammesunterschiede der verwendeten Versuchstiere, durch Ernährungsschäden, durch Fasten, durch Schwankungen in den Nährstoffbedürfnissen verschiedener Tiere und durch die Injektion gewisser Hormone beeinflußt werden können. Andere Faktoren, wie die placentare

Durchlässigkeit, die Anzahl der Jungen eines Wurfes und der mütterliche Stress während der Schwangerschaft können für die Beurteilung der teratogenen Wirkung im Tierversuch ebenfalls von Bedeutung sein.

Da der für die Mißbildungen verantwortliche, ursächliche Mechanismus bei Mensch und Tier gleichartig sein muß, sind die Ergebnisse von Tierversuchen für die Bewertung der Ursachen menschlicher Mißbildungen von beträchtlicher Bedeutung. Gebührende Beachtung muß jedoch den mannigfachen Faktoren geschenkt werden, welche die Resultate der Tierversuche beeinflussen können.

Résumé

En appliquant les expériences faites sur l'animal à l'homme, on doit être conscient que beaucoup de facteurs constatés dans l'expérience sur l'animal sont très différents pour l'homme. Les effets d'un agent tératogène suspect sont à évaluer contre un fond variable de la race, de l'environnement, du rang social, des habitudes diététiques, de l'infection, de l'état hormonal, de multiples grossesses et de troubles psychiques.

Beaucoup de chercheurs ont démontré clairement que la fréquence des malformations chez les placentalia et parfois même la nature de la déformation produite par l'emploi de substances tératogènes connues, peuvent être influencées par les différences de l'espèce, de la souche des animaux en expérience, par les troubles alimentaires, par le jeûne, par les variations des besoins métaboliques de différents animaux pour les substances nutritives et par l'injection de certaines hormones. D'autres facteurs, p. ex. la perméabilité placentaire, le nombre des petits et le stress maternel durant la gravidité peuvent aussi avoir de l'importance pour l'estimation des effets tératogènes sur l'animal en expérience.

Puisque le mécanisme causal responsable des malformations chez l'homme et les animaux doit être similaire, les résultats des expériences faites sur l'animal sont d'une importance considérable pour l'évaluation des causes des malformations humaines. Pourtant, les divers facteurs susceptibles d'influencer les résultats des expériences sur l'animal doivent être pris en considération.

Riassunto

Quando si applicano all'uomo le esperienze eseguite sugli animali, bisogna essere coscienti che molti dei fattori constatati nell'esperimento sulla cavia sono molto differenti per l'uomo. Gli effetti di un agente teratogeno sospetto sono da valutare con uno sfondo variabile della

razza, delle vicinanze, della posizione sociale, delle abitudine dietetiche, dell'infezione, dello stato ormonale, delle molteplici gravidanze e dei disturbi psichici.

Molti ricercatori hanno chiaramente dimostrato che la frequenza delle malformazioni nei placentalia e talvolta la natura stessa delle malformazioni provocate dall'impiego di sostanze teratogene conosciute, possono essere influenzate dalle diversità di specie, dall'origine degli animali esperimentati, da disturbi alimentari, dal digiuno, dalle variazioni dei bisogni metabolici dei diversi animali di sostanze nutritive, e dall'iniezione di certi ormoni. Altri fattori come per esempio la permeabilità placentare, il numero della prole, e lo stress materno durante la gravidanza possono anch'essi avere un'importanza nella valutazione degli effetti teratogeni sull'animale esperimentato.

Poichè il meccanismo causale responsabile delle malformazioni nell'uomo e nell'animale deve essere simile, i risultati ottenuti sugli animali sono di un'importanza considerevole per la valutazione delle cause di malformazioni umane. Però devono essere considerati quei diversi fattori capaci d'influenzare i risultati degli esperimenti sugli animali.

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