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TESTING DRUGS FOR TERATOGENICITY AND THEIR EFFECTS ON FERTILITY

The Present Position

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The intense activity stimulated by the discovery of the teratogenic effect of thalidomide has by now reached something like a plateau. It is now well established that many drugs in common use can produce fetal damage and affect fertility when tested in a variety of animal species. This includes a number of drugs that have been used for many years without any suggestion of a teratogenic effect and new drugs that have been discovered since the advent of thalidomide. Nevertheless, and fortunately, no new major teratogen in man has appeared, nor is there evidence of any gross interference with fertility as a side-effect of new drugs. This is thus a good time to look at all the evidence in order to determine whether we have indeed solved this problem of rejecting drugs potentially dangerous to the fetus and reproduction or whether we have just been lucky!

The most detailed methods of examining drugs for teratogenic action and for possible effects on fertility have been developed by the Food and Drug Administration (FDA) of the USA. Based on sound empirical reasoning, they try to take into account all possible known factors that might produce these effects. Though the FDA does not, of course, claim to be infallible, and does in fact set down guide-lines rather than impose specific techniques, the position nevertheless is that these recommendations are neglected, even in some of the details, at the peril of the pharmaceutical firms which are the main source of new drugs. We must, therefore, ask ourselves a number of questions regarding the FDA regulations.

i. Are they so framed as to give the maximum predictive value, which is, of course, the primary consideration? If so, the method will not only reject drugs which may prove to be toxic in these respects in man, it will pass drugs which will not be toxic in man though toxicity may be recorded in animals.

ii. Are the instructions too detailed and cumbersome, or could equally suitable regulations with the same degree of predictive value be framed with a simpler scheme of experiments?

iii. Have we neglected any approaches which might contribute to the value of the information obtained?

However confident we are that the most suitable methods have been established, rigorous reporting of toxic effects in man remains of paramount importance, so as to establish as rapidly as possible the occurrence of any toxic effects in man, which may not have been sufficiently accurately predicted by animal experiments. Though this has been said before it needs repeating: the use of new drugs whose potential hazards have not been fully established will depend to a great extent on the severity of the condition that is being treated. We will, for example, be much more ready to use them for malignant conditions than for the production of hypnosis or mild analgesia.

I will, therefore, give some details of the FDA regulations and then point out some important respects in which the British suggestions differ from the American ones. It is well realized that regulations have been formulated in other countries, but a detailed survey of these is not within the scope of the present paper. Rather do I aim to present a broad outline as a preliminary to discussion.

With regard to the FDA regulations, they are obviously much more detailed and onerous than the British notes, and it is my considered opinion that at present there is no evidence that the USA regulations will yield additional relevant results commensurate with the work involved.

1. Regulations of the Food and Drug Administration of the USA

The FDA regulations are fairly specific and aim at detecting the effects of drugs both on fertility (in males and females) and on the development of the fetus. Deleterious effects on the fetus may be observed during pregnancy and at birth, but in some instances may not develop until some time afterwards. Frequently all these experiments are carried out only in one generation, but if the same drug is used repeatedly over a long period embracing more than one generation, as in, e.g., the use of pesticides, then multi-generation studies should be carried out. It should be emphasized that these studies are essentially empirical and that, for example, no special attention is paid to the possible mechanism of action, or to the fate of the drug used in the body of the pregnant mother, or in the fetus (which may, of course, vary in different species). For this reason it is advisable that in these experiments, which are of a rather routine kind, the results should be scrutinized by a skilled and experienced investigator in the hope that a hint will be obtained of further possible lines of investigation. The problem of the translation of these results to man will be dealt with below.

The tests can be considered under three headings, namely:

i. study of fertility and general reproductive performance;
ii. teratological study; and
iii. perinatal and postnatal study.

These can be run simultaneously or sequentially.

The first series of tests have a wide scope and aim at giving general information on the effects of drugs on fertility in both sexes, the course of gestation, early and late stages of embryonic and fetal development, lactation and postnatal effects. They are usually and conveniently carried out in rats.

Young adult females are treated for about a fortnight before mating and treatment is continued until either they are killed or their offspring are weaned. Males are dosed, beginning at about six weeks of age, and treated for 10 or more weeks before mating (male animals from concomitant chronic toxicity studies may be used). The females are usually inspected daily and mating is indicated by the presence of sperm in the vagina or by copulation plugs; this is then day 0 of pregnancy.

One half of the mated females are killed on day 13 of established pregnancy and carefully examined: uterine contents are examined for number, distribution and condition of embryos, the presence of empty implantation sites indicating early embryonic loss; and any other abnormal condition of the uterus is noted.

The remaining dams are allowed to go to term (which occurs at about day 20–21) and to litter naturally. Observations are made on the duration of pregnancy, litter size, number and condition of dead pups and gross anomalies of the newborn. Since handling of the young should be kept to a minimum, they are weighed at birth and at 4 days and then again at 21 days. Any pups dying are carefully examined. In the light of the results so obtained it will be decided whether a second generation, or even more, should be investigated.

In the second series of tests possible teratogenic effects of drugs are investigated. At least two species should be used, and these are often chosen from mice, rats or rabbits (though other species deserve consideration, particularly monkeys, if suitable colonies could be established for this purpose). Animals are mated and the date of mating noted.

The drug is then given during the period of organogenesis, e.g. days 6–15 for rats and mice and days 6–18 for rabbits. Offspring are removed by Caesarean section one or two days before term, and live and dead fetuses, resorption sites and corpora lutea are examined and recorded. Fetuses are weighed and examined for gross anomalies. One-third of the fetuses are then dissected for detection of visceral anomalies and the rest are cleared and stained for skeletal abnormalities. In experiments on rabbits all fetuses should be examined for gross visceral and skeletal anomalies.

As already stated, the teratogenic effect of drugs administered during pregnancy may not become apparent until some time after birth, and these may include functional changes in the central nervous system.

The third series of tests are concerned with the detection of adverse effects on the dam and offspring produced by drugs during the perinatal and postnatal stage, i.e., by treatment during the last third of pregnancy and up to the period of weaning. Observations are made for delayed or prolonged labour, dystocia, lactation, maternal care and direct toxic action of the drug on the young. The presence of lactation is carefully established, particularly in cases of poor survival during the nursing period.

The official British and American points of view on the testing of drugs for teratogenesis and fertility are presented in comparative tables by Cook, Fairweather & Hardwick (1969), and there are many similarities between these suggestions. The practical interpretation seems to be rather different, the FDA being apparently much more rigid in this respect. Thus the Americans expect all three studies to be completed in order that a drug should be suitable for acceptance, i.e., reproduction, teratological and perinatal and postnatal studies. On the other hand, the United Kingdom's Committee on Safety of Drugs will accept a study of teratology alone, provided this has been done satisfactorily. In such a study the drug has to be given from day 1 until just before term, whereas according to the FDA it should be given during the period of embryogenesis, and thus damage produced by a drug during the later stage of pregnancy could be missed.

It is, however, probably worth while doing an initial experiment on small groups (6–8) of rats or mice, giving the drug in three different regimens, i.e., for days 0–20, days 6–15, and days 15–20. This will make it possible, from an examination of the fetuses removed by Caesarean section before parturition, to determine if the drug exerts an antifertility effect by an action before implantation, or exerts an antifertility effect on the embryo after implantation, or damages the mature fetus. This will help in the planning of the full teratogenic tests.

Another important difference between the British and American views is that three dose-levels are recommended in the British suggestions, whereas the FDA is satisfied with two dose-levels. Since there may be dose-levels that will kill the fetus and thus not reveal teratogenic effects, it appears to me that this is less likely to occur if three dose-levels are used, i.e., a high dose but not lethal to the mother, a low dose to produce a clinically relevant effect and an intermediate dose to be spaced logarithmically. The use of a logarithmic spacing is in agreement with that suggested by Robson & Sullivan (1968), in which publication a simple illustrative nomogram is given for the evaluation of such spacings of drug levels.

Controls

According to the British recommendations (see Committee on Safety of Drugs, 1968) there should be not only controls to establish the spontaneous occurrence of abnormalities in the species and strain used; in addition, evidence should be provided that the strain of animal used produces abnormal offspring after the administration of a known teratogen, such as thalidomide, during the gestation period.

2. Interpretation of Results of Studies of Teratogenesis

In general it can be said that the problems posed by the study of teratogenesis are essentially similar to those encountered in general toxicity studies and that the same basic principles can be applied. There is, however, an important practical difference concerned with the clinical use of the drugs. The patient who receives a drug, after its safety has been established in animal experiments, can be closely watched; and, if anything untoward occurs, the dose can be decreased, or administration stopped. In teratology, there are usually no such warning signs, and if any harm is done it may not be observed before many months have elapsed; hence the need for particular vigilance in the use of new drugs during pregnancy.

Much work has been done on the development of methods for the production and investigation of teratogenic effects in animals and a very large variety of substances have been shown to produce such effects. As already said, these include not only drugs of fairly recent origin, e.g. imipramine, but also substances which have been used for many decades in the treatment of diseases and which have been considered to be without deleterious effects on the human fetus. Salicylates offer a good relevant example of such a drug, but there are many others. Fortunately few of these drugs have produced abnormalities in man, but they include aminopterin, thalidomide, progesto-

gens and tetracyclines, with a few others under suspicion. This emphasizes the difficulties of using the data observed in animals as prognostic indices in man.

Let us first consider some circumstances in which a ruling could easily be made. If a particular drug has been given to at least two species of animals and has not produced any abnormalities, even in doses approaching the toxic level for the mother, then we shall be reasonably confident that the drug will be safe in man (although even then there is no absolute certainty). It is, however, worth realizing that an effective monitoring system has been developed for the recording of toxic effects, including abnormalities, so that should any drug be erroneously considered as safe on the basis of animal experiments, the damage in man will, we hope, be quite limited.

Assessment of the results of a second series of tests should also lead to a fairly confident appraisal. If a drug produces serious teratogenic effects in two species of animals in doses below those that produce serious toxic effects in the mother, then we will not accept it for clinical use—except possibly for the treatment of conditions so serious that it is worth trying anything. Thus, for example, such a drug would still be considered for the treatment of acute leukaemia if there were any indication that it might be of therapeutic value.

It is the drugs between these two extremes that pose the greatest challenge, and at present we do not possess methods by which their possible effect in man can be assessed with certainty. Some suggestions have, however, been made and it is worth examining them. One rather obvious idea is that the relation between the dose toxic to the mother (animal) and that which produces teratogenic effects on the fetus is of importance. Thus if the teratogenic dose of a drug is much smaller than that toxic to the mother, then this should suggest that the drug will be harmful to the fetus, and vice versa. This relationship has been expressed as the teratogenic ratio, i.e., the ratio between the LD_{50} for the mother and the dose which is teratogenic to the fetus. Quite arbitrarily, it has been suggested (Robson, 1963; Robson & Sullivan, 1968) that, if the ratio is greater than 10, then the drug should be rejected. The idea has been worked out in some detail and from preliminary results it would appear that the use of the teratogenic ratio has some validity (Robson & Sullivan, 1968). It certainly would have rejected the main human teratogens. The reader is referred to the paper by Robson & Sullivan for a more detailed discussion, including the actual data.

Another ratio which has been considered is that between the teratogenic dose in animals and the therapeutic dose in man; it has been suggested that the smaller the ratio, the greater the fear of a toxic effect on the fetus. An analysis of some results (see Robson & Sullivan, 1968) does not suggest that this approach will be a fruitful one.

The margin between the embryotoxic dose (that which will kill the fetus) and the teratogenic dose has also received consideration (Tuchmann-Duplessis, 1965), and it seems reasonable to consider that the larger the margin is, the more dangerous the compound is likely to be. Often, however, the two effects are not easily separable, so that fetal deaths and malformations are observed at all doses tested.

One last important point needs to be made in this discussion. A large number of data relating to various compounds are now being accumulated by the agencies that control the testing and the use of drugs. It may well be that already sufficient information has been collected to allow definite answers to the question of interpretation raised above; e.g. to what extent do the data on teratogenic effects of new drugs correlate with the various data referred to above? Unfortunately, at present, to the best of my knowledge, these data are not being used for this purpose. For obvious security reasons, they are not available to workers outside the agency that is making the investigation, and the agencies themselves are essentially concerned with giving rulings on data and not with conducting research. It is earnestly to be hoped that some mechanism will be devised by which an analysis of the accumulating material will become possible, leading to a clearer interpretation of results obtained in animals.

3. Some Recent Problems

a. Lysergic Acid Diethylamide (Lysergide; LSD 25)

There is a fair amount of experimental work on animals which shows that LSD 25 is a teratogen and can also interfere with fertility. Thus, for example, Geber (1967) showed that the drug would produce malformations, particularly of the central nervous system in hamsters, when given in very small doses. Malformations were also produced by mescaline and 2-bromo-LSD (BOL 148), and all three drugs caused an increase in resorption and fetal mortality.

The production of abnormalities with LSD 25 has also been observed in mice, again with very small doses equivalent to a single dose given in man (Auerbach & Rugowski, 1967). Various effects on pregnancy and on the fetuses have also been reported in rats (Alexander, Miles, Gold & Alexander, 1967).

There are also a number of reports of LSD 25 producing various types of damage to chromosomes, both in vitro and in vivo. The significance of this work is difficult to evaluate. In the first place, Egozcue, Irwin & Maruffo (1968), who investigated these effects in patients who had taken LSD 25, were unable to find a dose–response relationship between the dose of the drug and the frequency of chromosomal breaks. It is also worth remembering that exposure to x rays for diagnostic purposes will induce a similar increase in chromosomal breaks, as will some viral infections and exposure to a variety of other drugs (see Zellweger, McDonald & Abbo, 1967). It would, of course, be interesting to know what effects are produced by LSD 25 in monkeys. As for man, there are suggestive reports of the occurrence of limb abnormalities in infants of women who had taken LSD 25 at a time critical for the formation of limb deformities (Zellweger *et al.* 1967; Assemany, Neu & Gardner, 1970).

It is, therefore, worth while examining LSD 25 in the light of some of the criteria we have discussed. According to data in the literature the LD_{50} of LSD 25 is of the order of 54 mg/kg, while the teratogenic dose is very small, i.e., 0.1 μg/kg. This gives a very high teratogenic ratio of over 500,000 and on this basis the drug should certainly be rejected. The other ratio considered, i.e., the teratogenic dose over the therapeutic dose, gives a value of about 0.01—again leading to a rejection of the drug. Both these criteria lead to a poor prognosis, and it is to be hoped that we shall never have a clinical confirmation of this.

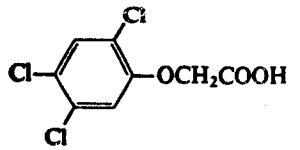
b. Other Drugs

There is evidence that one form of cannabis (ganja) can produce serious teratogenic effects when administered to rats (Persaud & Ellington, 1968). However, suitable data are not

available for doing the calculations that have been performed for LSD 25. It is highly desirable that such data should be obtained, since cannabis is used quite extensively in a population which includes a fairly high proportion at an early stage of pregnancy.

c. Chemical Defoliants

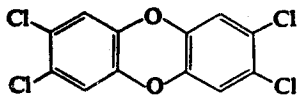
Two such substances have been investigated, namely, 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) (I) and 2,4-D (2,4-dichlorophenoxyacetic acid). They are particularly interesting as they have definitely been shown to be teratogenic in animals (rats and mice) (T. W. Edminster, personal communication, 1970). It has also been claimed that they have produced teratogenic effects in man (in Vietnam), though this has by no means been established. In fact, it has been stated that there is no information that 2,4,5-T would cause adverse fetal effects in man (T. W. Edminster, personal communication, 1970). It is thus worth while examining data quantitatively to see whether these would yield any additional information. The LD_{50} of 2,4,5-T, given to rats orally, is 300mg/kg (*The Merck Index*, 8th ed., 1968) and the minimal teratogenic dose in rats, also when given orally, is 4.6mg/kg (McCrystal, 1969). The teratogenic ratio, as defined above, is thus 65, which is well above the value of 10 arbitrarily chosen as the minimal value denoting a potential risk of teratogenic effect of a drug in man. On this basis, therefore, this substance would thus be expected to be a teratogen in man.



2,4,5-trichlorophenoxyacetic acid

I

The problem is complicated by the fact that the sample of 2,4,5-T used in the investigation for teratogenicity by the Bionetics Research Laboratories, Bethesda, Md, contained about 27p.p.m. of a toxic contaminant, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (II), which arises in the process of manufacture and has an LD_{50} in rats of 0.022–0.045mg/kg. More recent samples of 2,4,5-T produced by the Dow Chemical Company have less than 0.5p.p.m. of dioxin and have not produced teratogenic effects in rats and rabbits, when administered in doses up to 24mg/kg and 40mg/kg respectively; and this is reassuring. At present the dioxin problem is under intensive investigation in the Department of Agriculture and the Department of Health, Education and Welfare in the USA (T. W. Edminster, personal communication, 1970). Further data are given in a contribution to *Nature* (1970) but it still leaves the question open.



2,3,7,8-tetrachlorodibenzo-*p*-dioxin

II

The problem of the chemical defoliants has been discussed in some detail since it raises acutely the relevance of animal experiments in the interpretation of drugs which may be potentially teratogenic in man. Obviously further work is required, but in the meantime it would seem wise to limit, or even to ban, the use of these drugs in situations where they might affect pregnant women.

4. Summary

It is obvious from the above that methods for detection of drugs producing congenital abnormalities have been put forward by various agencies. Essentially the methods are empirical and consist of administering drugs at various dose-levels to animals and examining fetuses not only by the naked eye, but also for soft-tissue and bony anomalies. Under these conditions a large number of drugs have proved to be teratogenic, mostly when given in doses toxic to the mother, and the problem is one of interpreting these results and also of devising methods which would be more selective. A beginning has been made in devising quantitative approaches to the problem, and this needs further investigation in the light of data which are being accumulated, mostly by various agencies. All this, however, will still represent only an empirical approach, and it is quite likely that nothing more is possible at present.

What is obvious, from our knowledge of general toxicity testing, is that investigations on the metabolism and elimination of drugs, as well as on the passage of these drugs and their metabolites into the embryo, should ultimately form an essential part of the investigation on fertility and teratogenesis. This would help in deciding to what extent results obtained in various species are applicable to man, and to select the particular animal species most similar to man in the way it deals with the drug under consideration.

It has been suggested that monkeys would probably represent the optimum species in the majority of cases, and that colonies of such animals should be set up for this purpose, and it is interesting that thalidomide has produced in this species abnormalities of the types seen in man (Delahunt & Lassen, 1964). The cost would be prohibitive if such colonies were used for all the routine testing, but there is no reason why they should not be invoked when tests on lower animals have given equivocal results. In this connexion, however, the recent history of an oral contraceptive, chlormadinone, appears to be anomalous. This drug has apparently produced some kind of tumours, though non-malignant, in the mammary glands of bitches after prolonged administration, though it seems to be free of such toxic effects in monkeys; on the basis of this result chlormadinone has been withdrawn by the manufacturers. Unless there is clear evidence that the fate of this drug in the body of the bitch is more akin to man than is its fate in monkeys, this seems an illogical decision, suggesting that the use of monkeys will not represent any advantage for the testing of drugs for various types of toxicity.

Finally, nothing has so far been said about the mechanisms by which drugs produce their embryotoxic and teratogenic effects, though a fair amount of work has been done on thalidomide. There is good evidence that a variety of mechanisms are involved with different drugs, e.g. inhibition of mitotic processes with aminopterin, or the effective level of the hormone necessary for the maintenance of normal pregnancy, or insufficient blood and oxygen supply to the embryo. Much more basic work on the mechanisms of action of such drugs is necessary.[1] At present spectacular work is being done on the nature of receptors through which substances naturally present

[1] See also "Mechanisms of Toxicity" (*Br. med. Bull.* 1969, vol. 25, no. 3).—ED.

in the body as well as on the way drugs foreign to the body produce their effect. It may well be that similar techniques should be applied to the problem of the action of substances on the embryo and on pregnancy.

In this connexion the finding of Wilk, Steffek & King (1970) that the teratogenic activity of norchlorcyclizine derivatives varies with their ability to bind with cartilage, in vitro, is of interest.

References

Alexander, G. J., Miles, B. E., Gold, G. M. & Alexander, R. B. (1967) *Science, N.Y.* **157**, 459

Assemany, S. R., Neu, R. L. & Gardner, L. I. (1970) *Lancet*, **1**, 1290

Auerbach, R. & Rugowski, J. A. (1967) *Science, N.Y.* **157**, 1325

Committee on Safety of Drugs (1968) *Notes for the guidance of manufacturers and other persons developing or proposing to market a drug in the United Kingdom.* Committee on Safety of Drugs, London

Cook, M. J., Fairweather, F. A. & Hardwick, M. (1969) In: Bertelli, A. & Donati, L., ed. *Teratology*, p. 34. (Proceedings of a symposium organized by the Italian Society of Experimental Teratology, Como, 21–22 October 1967.) Excerpta Medica Foundation, Amsterdam & London

Delahunt, C. S. & Lassen, L. J. (1964) *Science, N.Y.* **146**, 1300

Egozcue, J., Irwin, S. & Maruffo, C. A. (1968) *J. Am. med. Ass.* **204**, 214

Geber, W. F. (1967) *Science, N.Y.* **158**, 265

McCrystal, C. (1969) *Sunday Times*, 30 November, p. 7

Nature (1970) **226**, 309

Persaud, T. V. N. & Ellington, A. C. (1968) *W. Indian med. J.* **17**, 232

Robson, J. M. (1963) *Proc. R. Soc. Med.* **56**, 600

Robson, J. M. & Sullivan, F. M. (1968) In: Boyland, E. & Goulding, R., ed. *Modern trends in toxicology 1*, p. 86. Butterworths, London

Tuchmann-Duplessis, H. (1965) In: Robson, J. M., Sullivan, F. M. & Smith, R. L., ed. *Embryopathic activity of drugs*, p. 56. (Biological Council symposium.) Churchill, London

Wilk, A. L., Steffek, A. J. & King, C. T. G. (1970) *J. Pharmac. exp. Ther.* **171**, 118

Zellweger, H., McDonald, J. S. & Abbo, G. (1967) *Lancet*, **2** 1066

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