Environmental Health Perspectives
Vol. 30, pp. 99–103, 1979

Topical Problems and Basic Developmental Trends of Investigations Concerning the Embryotoxic and Teratogenic Effect of Environmental Chemicals

by A. P. Dyban*

The development of effective methods for prevention of embryo disorders and congenital defects appears to be one of the cardinal tasks of modern medicine. The development of man and the health of future generations depends upon a successful solution to this complex problem. The etiology of congenital defects in humans is multifactored since they occur as a result of the injurious effect of environmental agents (so-called teratogenic agents) or because of genetic factors (mutant genes and chromosome aberrations). They also occur as a result of the combined effects of teratogenic agents and genetic factors. The latter is the most frequent etiology of developmental abnormalities.

It follows then, that first there exists the necessity of studying the embryotoxic and teratogenic effects of various environmental factors. Secondly, there is the advisability of conducting investigations in an uninterrupted sequence with analysis of the genetic factors facilitating the occurrence of the teratogenic effect.

It should be emphasized that the effect on embryogenesis of mammals and humans (with different external agents) has not been studied to the same extent. Extensive information has been accumulated concerning the teratogenic effects of ionizing radiation and pharmacological substances, but the teratogenic properties of chemical components of environmental pollutants have not been studied (in animals) sufficiently. Many of these chemical substances have not yet been tested on embryos, and other experiments have yielded insufficient data. In addition, persuasive information has been received, from USSR–US collaborative efforts (*1, 2*) that is indicative of the teratogenic and embryotoxic effect of several pesticides and industrial poisons (*3, 4*).

Hence, the presence of potential teratogenic agents in environmental pollution can no longer be ignored; but which of these factors presents a danger to human embryogenesis? It is necessary to learn how these factors influence embryogenesis; if not for all of them, at least for those chemical contaminants most frequently found in environmental pollution. However, the number of chemical substances is extremely large and is rapidly growing. The routine methods of testing for teratogenicity are extremely laborious and very time-consuming. To screen for teratogenicity by means of present-day methods for just the basic chemicals of environmental pollution would require the efforts of a large number of laboratories and would require considerable material resources. It is impossible to solve this problem in the immediate future.

It seems doubtful whether it is worthwhile to test all the chemicals of the environment in sequence. Rather it would probably be more promising and expedient to improve prediction of teratogenic chemicals to study the mechanisms of the induced teratogenesis. For instance, the derivatives of 2,4 diaminopyrimidines demonstrate a close association between teratogenic activity and specific features of chemical structure. Teratogenic activity regularly fluctuated as a function of the molecular structure (*5, 6*), and was accurately correlated with

* Department of Embryology, Institute of Experimental Medicine, U.S.S.R. Academy of Medical Sciences, Leningrad, U.S.S.R.

capacity to depress the enzyme dihydrofolic reductase (*7*) and antimitotic activity (*8*). Examples of associations between teratogenic activity and chemical structure of pharmacological substances are well known (*9*).

The study of molecular mechanism, teratogenic effects, and the association of these properties with molecular structure are of considerable interest, not only for providing knowledge of the mechanisms of induced teratogenesis, but also for revealing methods of predicting teratogenic activity of environmental chemicals. An urgent necessity exists to apply more adequate methodological approaches for testing the teratogenicity and embryotoxicity of chemical substances.

We should note that the methods currently used for studying the effect of the chemical environment on embryogenesis were originally devised only for testing embryos for the effects of new drugs (*10*). They test the teratogenicity of drugs on the basis of fundamental pharmacodynamic data; in addition, various dosages of the drug (from toxic to therapeutic) are administered to female animals (*11, 12*) on a single day or on several days. Such testing is not only not predictive, but also cannot reveal the consequences of small dosages of chemical substances (*11*).

It is necessary to emphasize that teratogenic agents have not been studied sufficiently in regard to dosage-time effect relationships. Instances are well known, in which the teratogenic agent causes developmental abnormalities only after single-day administration of the drug and not after chronic administration to the female (*3, 13*). It is necessary to implement special and well thought-out experiments with different types of chemical teratogenic agents using various routes of administration and various durations of exposure to study the mechanism of this phenomenon. This appears to be a complex task, the solution of which in many ways is dependent on the development of more reliable methods for evaluating the teratogenicity of those chemical substances which chronically influence the human body.

In addition, it is extremely important to investigate the effect on mammalian embryogenesis of several teratogenic agents administered simultaneously at a different range of doses. The human body frequently undergoes multiple attacks of several environmental agents, each of which has potential teratogenic activity. Studies in this direction are only beginning, and considerable effort is required to define which of the chemical agents are teratogenic and to determine the conditions required for synergistic activity.

Experimentally the teratogenic effect of several chemical substances is increased due to stress and altered maternal status (*14, 15*) and also to incomplete protein supply and hypovitaminosis (*16*). Further investigations are necessary to establish whether this phenomenon concerns only specific teratogens or is more widespread in character.

It is a necessary condition of the standard teratologic experiment to use a genetically uniform group of healthy animals at an age when reproductive function is optimum. However, if the teratogen contaminates our environment, populations of various age groups are likely to be exposed, including both people with a hereditary and predetermined increased sensitivity to a given substance and also patients and weakened individuals.

Numerous experiments indicate that the reaction to several chemical teratogens fluctuates significantly as a function of the genotype of the female and of the fetus (*3, 17*). The causes of this phenomenon are not yet entirely clear; they include different rates of embryonic development, close to the sensitive links of the animals which are predetermined features of teratogenic metabolism in the mother's body and in the tissues of the fetus. There are also the differences in the sensitivity of the same embryonic rudiments to the teratogen. This contemporary question needs further study through use of different chemical teratogens.

In the presence of several latent infections of the female, the frequency of embryo mortality and abnormalities of development increases (*18*). This problem also deserves a deeper analysis.

Correct evaluation of the danger of chemical substances to human embryogenesis is dependent upon study of the influence of a series of accompanying factors on the teratogenic effect. It is important to clarify and expand the criteria utilized for judgments concerning the harmful effect of the foreign agents on embryogenesis.

During the routine testing of chemical substances on fetuses, usually fetal mortality and morphological variations of the surviving fetuses are taken into account (*3, 10–12*). These ordinary indicators make it possible to obtain significant information about embryotoxic and teratogenic effects. However, embryonic death rate and morphological abnormalities of the fetus do not in the least exhaust all of the injurious effects of exogenous agents on embryogenesis. Teratogens also induce functional and biochemical abnormalities. These types of effects are now being actively investigated and initial results are extremely promising. Usually the obvious findings are obtained in the so-called "teratology of behavior;" that is, it is demonstrated that the teratogens can induce significant abnormalities in the higher neural activity of animals (*19, 20*). Evi-

dently, it is now time to think about the initial behavioral tests involving a number of criteria concerning induced teratogenesis. But before recommending this, as one of the compulsory stages in the testing of chemical agents for teratogenicity, it is necessary to apply considerable effort to the selection of an animal species and to standardization of the higher nervous alterations which are likely to be useful in routine teratological experiments. This presents a broad field of activity for possible collaboration among embryologists, physiologists, and behavioral geneticists.

We know that if animals are exposed prenatally to several chemical teratogenic agents, abnormalities of the reproductive system can occur due to damage of the hypothalamus, hypophysis, or the gonads. This trend of investigation deserves proper attention and further development. In this case if the teratogen damages the germ cells, the boundary between teratogenic and mutagenic effects can be erased. Therefore, during teratological analysis it is necessary to study how teratogenic agents affect development of the gonads and to attempt to differentiate this effect from the capacity of the teratogen to induce chromosome abnormalities (or mutations in the gametes).

Significant interest demands a comparison of the effect of teratogens on spermatogenesis and oogenesis during the prenatal and postnatal stages of development. The increased sensitivity found at several stages of oogenesis indicate that small dosages of teratogens induce chromosomal aberrations which then are passed on to the fetus (*21*). Alterations of the chromosomal apparatus produced in oocytes and zygotes may serve as a highly responsive supplementary criterion for identification of the injurious effect of chemical teratogens. Such broadening of the criteria of teratogenicity is important to address the practical question of how to determine the cut-off points and the maximum safe dosages for chemical teratogens. Naturally, for a reliable determination of these dosages it is necessary to employ not only indicators of embryonic mortality and abnormalities of organogenesis, as revealed by morphological methods, but also to employ a number of other (biochemical, physiological, behavioral, cytogenital, etc.) criteria more able to characterize the harmful effects of chemical substances on embryogenesis. However, as some of these criteria are more sensitive than others, special studies with several classes of teratogens are required. Such studies should provide a basis for determining approaches for analyzing the cut-off point between teratogenic and non-teratogenic dosages of chemical teratogens.

Apparently teratological experiments can also play a significant role in the solution of this problem. This course of study recently has begun to develop rapidly (*22, 23*). Use of mammalian fetuses developed *in vitro* or of organ cultures permit detection of subtle mechanisms of teratogenic activity operating directly upon the differentiating embryo as opposed to effects operating through placental alterations or through alteration of the pregnant female. These methodological procedures make it possible to speculate concerning the period of time of teratogenic activity when abnormalities of embryogenesis are reparable or are irreversible.

Examination of these interesting questions are beyond the scope of this report. It follows only to emphasize that the value of *in vitro* studies for solving theoretical questions in teratology cannot be doubted. However, there is not a concensus among teratologists regarding the advisability of employing these methodical procedures for testing the teratogenicity of chemical substances. For example, in *in vitro* cultures of preimplantation mouse, rat, and rabbit embryos, it was not possible to reveal the injurious effect of thalidomide and dimethyl sulfoxide (*24*), but antifolic preparations (in small doses) showed a pronounced injurious effect on preimplantation mouse and rat embryos (*25, 26*) and also on post-implantation rat fetuses cultivated *in vitro* (*27–29*). Therefore, for several classes of teratogenic agents, mammalian fetuses being developed *in vitro* are undoubtedly more sensitive than the fetuses being developed *in vivo*. However, further study of this question is needed and it is important to compare the response of a series of systems (being developed *in vitro*) to different teratogens.

Talk can continue about cultures of preimplanted embryos of mice and rats, cultures of implanted rat and mice fetuses, also about organ cultures of the hard palate and the limb buds, but it is necessary to determine exactly which of these systems gives the most reproducible results and which can be recommended for testing the embryotoxicity and teratogenicity of chemical substances.

Because *in vitro* teratological experiments require considerably less time than *in vivo* tests, potential use of these approaches for preliminary screening of chemical substances, that is, the analysis of their embryotoxicity (*24*), is extremely attractive. *In vitro* tests also may be used for the analysis of the cut-off points between teratogenic and non-teratogenic dosages of teratogens.

The study of mechanisms of specific differences in reaction to teratogens remains extremely up-to-date. This feature is firmly established by numerous observations and can be checked by the overall regularity of the induced teratogenesis. However,

mechanisms of specific differences in teratogenic effect are not studied sufficiently. In addition to differences in response due to comparative toxicology (the differences in the metabolism of the teratogens, their distribution to the blood and organs of the female and fetus, detoxicants, etc.), there are also differences in response due to purely embryological factors, that is, the features intrinsic to the development of the fetuses of the various mammals which play a significant role in the given phenomenon.

For example, in the embryogenesis of marsupials and rodents (the favorite subjects of tests for teratogenicity and embryotoxicity), the omphalitic stage of placentation occurs and inversion of the embryonic layers takes place. These processes are not found in the embryogenesis of many other mammals. Therefore, expanded studies of comparative embryology and teratology of different mammals are advisable in connection with the study of teratogenicity and embryotoxicity of chemical substances.

It is known that in solving a specific teratologic problem, fetuses of a certain animal species can be more suitable than fetuses of another species. However, it is doubtful if an animal species will be found whose fetuses react to teratogens as do human fetuses. In addition, attached to the decision to employ additional species of animals in teratologic experiments, one necessarily must face many questions such as: Can the species be bred commercially under laboratory conditions? Has their normal embryogenesis been fully studied? What advantages are to be gained over use of more commonly used species like rats, mice, and rabbits that are broadly adopted for studies of embryotoxicity and teratogenicity?

Conclusion

In this report, problems are examined which must be solved to enhance the medical significance of results of teratologic experiments on chemical contaminants of the environment, and to facilitate the extrapolation of experimental findings to human embryogenesis. To determine the minimal effective and maximal nonfunctional dosages of teratogens and to consider the question of their maximum permissible concentrations in the environment, it is necessary to improve considerably methods of testing for teratogenicity. The criteria for teratogenicity should be broadened, a wider range of biological models should be used which include fetuses being developed *in vitro*, and factors influencing teratogenic activity should be determined. The basic classes of chemical teratogens should be studied in relation to dose-time effect and its role in regard to results after different routes of entry into the organism, and a series of other factors.

REFERENCES

1. Martson, L. V., and Voronina, V. M. Experimental study of the effect of organophosphoric pesticides on embryogenesis. In: Information from the First Soviet-American Symposium on the Problem Environmental Health, Moscow, 1975, pp. 168-172.
2. Staples, R. E., Kellam, R., and Haseman, T. K. Different toxic effects on rats during pregnancy after the administration of organophosphoric pesticides to the stomach. In: Information from the First Soviet-American Symposium on the Problem Environmental Health, Moscow, 1975, pp. 173-183.
3. Wilson, J. Environment and Birth Defects, Academic Press, New York-London, 1973.
4. Shepard, T. Catalog of Teratogenic Agents, Hopkins Univ. Press, Baltimore-London, 1973.
5. Khomov-Borisov, N. V., Dyban, A. P., Tikhodeeva, I. I., and Chebotar, N. A. Characteristics of chemical structures of analogs of anti-malarial specimens of chloride (pyrimetamine), having determined their teratogenic characteristics. Pharmakol. Toksicol. 3: 341 (1976).
6. Dyban, A. P., Barilyak, I. R., and Tikhodeeva, I. I. Teratogenic and embryotoxic effect of several derivatives of 2,4-diaminopyrimidine. Arkh. Anat. Gistol. Embriol. 71: No. 7, 29 (1976).
7. Rostovtsev, V. N. Study of dihydro-folic reductase in normal embryogenesis and during induced teratogenesis. Thesis, Leningrad, 1974.
8. Dyban, A. P., Barilyak, I. R., Tikhodeeva, I. I., and Chebotar, N. A. About the correlation of the teratogenic and anti-mitotic effect of a series of derivatives of 2,4-diaminopyrimidine. Ontogenesis 7: No. 1, 58 (1976).
9. Dyban, A. P., Tikhodeeva, I. I., and Khromov-Borisov, N. V., The association of teratogenic activity with chemical molecular structures of medicinal substances, Vestn. Akad. Med. Nauk SSSR 12: 78 (1975).
10. WHO. Principles for the Testing of Drugs for Teratogenicity, Tech. Rept. Ser. No. 364, WHO, Geneva, 1967.
11. Dyban, A. P. Basic approaches to the study of embryotoxicity and teratogenic properties of chemical substances. In: Information from the All-Union Conference on the Hygiene and Toxicology of Pesticides. Kiev, 1969, pp. 128-39.
12. Dyban, A. P., Borisov, V. S., and Akimova, I. M. New methodical approaches to testing teratogenic effect of chemical substances. Arkh. Anat. Gistol. Embriol. 59: No. 1, 89 (1970).
13. Dyban, A. P. Several topical problems of experimental teratology. Vestn. Akad. Med. Nauk SSSR, No. 1, 18 (1967).
14. Chebotar, N. A. Characteristics of the effect of salicylate, on different stages of embryogenesis of rats and rabbits: its influence on several shifts in the female. Farmakol. Toksikol. 30: 221 (1967).
15. Chebotar, N. A. Analysis of the mechanisms of the harmful effect of sodium salicylate on the embryonic development of rats. Thesis, Leningrad, 1970.
16. Dyban, A. P., and Akimova, I. M. Study of group B vitamins and genetic factors in the reactions of rat embryos to thalidomide. Arkh. Anat. Gistol. Embriol. 51: No. 8, 3 (1966).
17. Fraser, C. Some genetic aspects of teratology. In: Teratology, Principles and Techniques. J. Wilson and J. Warkany,

Eds., Univ. Chicago Press, 1964, p. 1.
18. Röhborn, G., Mutagenicity tests in mice. I. The dominant lethal method and the control problem. Humangenetik 6: 345 (1968).
19. Barlow, S., and Sullivan, F. Behavioral teratology. In: Teratology Trends and Application. C. Berry and D. Poswillo, Eds. Springer Verlag, New York, 1975.
20. Langman, J., Rodier, P., Webster, W., Crowley, K., Cardell, E., and Pool, R. The influence of teratogens on cellular and tissue behavior during the second half of pregnancy and their effect on postnatal behavior. In: New Approaches to Evaluation of Abnormal Embryonic Development. D. Neubert and H. Merker, Eds., Thieme, Stuttgart, 1975, pp. 439-468.
21. Dyban, A. P. Cytogenetics of initial embryogenesis in mammals. Vestn. Akad. Med. Nauk SSSR, No. 1, 18 (1973).
22. Kochhar, D. The use of *in vitro* procedures in teratology. Teratology 11: 273 (1975).
23. Neubert, D., and Merker, B. In: New Approaches to the Evaluation of Abnormal Embryonic Development, D. Neubert and H. Merker, Eds., Thieme, Stuttgart, 1975.
24. Staples, R. Potential of direct application techniques for detection of teratogens. In: New Approaches to Evaluation of Abnormal Embryonic Development. D. Neubert and H. Merker, Eds., Thieme, Stuttgart, 1975, pp. 71-81.
25. Dyban, A. P., Sekirina, G. G., and Golinskiĭ, G. F. Responses to anti-folic chloride specimens of early fetuses of mice. Bull. Exptl. Biol. Med. 82: No. 10, 1251 (1976).
26. Dyban, A. P., Sekirina, G. G., and Golinskiĭ, G. F. Effect of aminopterin preimplanted rat embryos cultured *in vitro*. Ontogenesis 8: 121 (1977).
27. Popov, V. B. Study of harmful effect of chloride in early post-implanted stages of different rat embryos (tests *in vivo* and *in vitro*). In: The Role of External Environmental Factors in Ontogenesis, Moscow, 1974, p. 35.
28. Popov, V. B. Cultivation *in vitro* of post-implanted mammal embryos. Ontogenesis, in press.
29. Popov, V. B., and Puchov, V. F. Comparison of the effect of chloridin on rat embryos in postimplantation stages of development in *in vivo* and *vitro* tests. Ontogenesis 8: 76 (1977).