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DRUGS AS TERATOGENS IN ANIMALS AND MAN[1,2,3]

By David A. Karnofsky[4]

Divisions of Clinical and Experimental Chemotherapy, Sloan-Kettering Institute, Department of Medicine, Memorial and James Ewing Hospitals, New York, N. Y.

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It has been stated that "any drug administered at the proper dosage, and at the proper stage of development to embryos of the proper species—and these include both vertebrates and invertebrates—will be effective in causing disturbances in embryonic development" (1). Since embryonic cells are proliferating rapidly, they may be unusually sensitive to the direct action of certain drugs or to environmental changes produced by the drug. During organogenesis, injury or destruction of specific cells may be manifest later as derangements in forms or in function within the context of a viable organism. The end result will deviate from the normal pattern of differentiation and maturation of the individual, as ordained by the genotype at the time of fertilization.

The vast array of available drugs has been shown to act on every aspect of cell structure and function. Depending on the species and, in some cases, on the strain, the embryo may be affected directly or through an altered maternal environment. The stage of embryonic development when a drug is administered is critical in determining the sensitivity of the embryo and the pattern of embryonic abnormalities. The expression of a developmental defect may be morphological or functional and may be evident early in development, in the perinatal period, or at various postnatal periods. Drugs which produce teratogenic disturbances in mammals are likely to produce fetal death and resorption when given at higher doses (2).

A study of the actions of drugs on embryonic development has ramifications into all fields of chemistry and biology. The variety of species of embryos used by investigators makes it difficult to establish general principles. Specific observations are only of interest to the naturalist unless analyzed in a penetrating manner; these may involve the relationship of the mechanism of action of the drug to specific biochemical pathways in

[1] The survey of literature pertaining to this review was completed in August, 1964.

[2] The following abbreviations are used: DNA (deoxyribonucleic acid); RNA (ribonucleic acid); thioTEPA (m, m′, m″-triethylenethiophosphoramide); DON (6-diazo-5-oxonoreucine); FCdR (5-fluorodeoxycytidine); FUdR (5-fluorodeoxyuridine); IUdR (Iodedeoxyuridine); 5 BUdR (5-bromodeoxyuridine); ClUdR (chlorodeoxyuridine); 3-AP (3-acetylpyridine); and 6-AN (6-aminonicotinamide).

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vulnerable cells, effects of the drug on the maternal host, a search for possible differences in the genotype of the mother and the fetus, developmental functions of the damaged cells as disclosed by an abnormal pattern of differentiation, and, finally, reconstruction of the observed developmental defects at various stages of gestation in an attempt to define the primary lesion caused by the drug.

Teratology did not evolve as a broad and well-balanced discipline; various groups adopted its methodology in order to accomplish their particular objectives. The morphologists were dominant for many years, describing in classical monographs the variety of birth defects occurring in animals and man (3, 4). The approach of descriptive teratology was subsequently broadened by experimental embryologists and geneticists. Investigators became interested in developmental mechanisms—interaction of cells, differentiation, molding of structures, development of form, growth of the organism, and expression of genetic traits. General principles were sought among the almost infinite variety of organisms available for study. Drugs were used as tools to dissect developmental mechanisms by relating the action of a drug on a biochemical process to the resultant developmental effect. This approach is illustrated by the recent work on the effects of actinomycin D which blocks DNA-dependent RNA synthesis probably by inhibition of messenger RNA (5) and interferes with differentiation and embryonic development (6, 7). The experimental embryologist is using alkylating agents, steroid hormones, antimetabolites, antibiotics, and vitamin excesses and deficiencies to study fundamental problems in embryogenesis.

During the past 30 years, specific drugs were found to produce consistent teratogenic effects in the appropriate species (8), usually pregnant mice and rats and chick, frog, and echinoderm embryos.

Developmental defects in the human fetus are of great concern to pediatricians, obstetricians, orthopedists, pharmacologists, and epidemiologists, among others. In recent years, the discoveries that birth defects were associated with a maternal rubella infection and that progestational hormones when administered to the mother had virilizing effects on the fetus attracted only limited public attention. The disjointed and rather placid field of teratology was shaken in 1962 by the thalidomide disaster. Biochemists, pharmacologists, embryologists, geneticists, and epidemiologists were called upon to explain the unanticipated effect of thalidomide on the human fetus. A scientific problem was posed in its most poignant and urgent terms in maimed, but surviving, innocent infant victims of exposure to a drug that had been regarded as almost innocuous in man. The result has been a rapid expansion in teratological research.

The post-thalidomide teratology program has two practical objectives: (*a*) to determine how thalidomide acted on the human fetus; and (*b*) to devise reliable preclinical methods to evaluate the teratogenic potentiality of a drug for the human fetus. The latter purpose is not to eliminate from

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clinical use a drug with teratogenic properties but is, rather, to estimate the hazard its use presents to the human fetus. Drugs with known teratogenic activity are used in patients with various forms of cancer; the therapeutic necessity for their use, however, outweighs the chance hazard that a drug might be given to a patient during early pregnancy and might exhibit a teratogenic effect.

This review will be concerned with some practical aspects of the relationship between drugs and the developing embryo.

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Current Sources of Information

An extensive literature, largely post-thalidomide, is available on the effects of drugs on embryonic development with particular emphasis on their potential toxicity to the human fetus. A succession of volumes containing the proceedings of organized symposia on birth defects have appeared in the past 5 years (9, 10, 11, 13, 14, 17). While there is considerable overlapping in the material covered, the problems related to develop mental abnormalities are broadly discussed.

Following the thalidomide disaster, the independent Commission of Drug Safety was established in the United States; and the recommendations of its Subcommittee on Teratology have been reported (18). In furthering its recommendations, the Commission sponsors a workshop in teratology; and the proceedings of the first workshop have been published (19).

The *Proceedings of the Congenital Anomalies Research Association of Japan* (20, 21) and abstracts of annual meetings of the Teratology Society in the United States are available (22, 23). Abstracts of the *Current Literature on Congenital Anomalies* were published by the National Foundation during the period 1960 to 1962 (24); this service was temporarily interrupted until 1964, to be replaced by *Birth Defects: Abstracts of Selected Articles* (25). A review of teratology was published for 1962 (26), and a yearly abstracts review is planned.

There are numerous excellent articles on the factors producing developmental disturbances in laboratory animals (8, 27–41, 103). The transport of various materials across the placenta has been reviewed (42, 43, 44); and Done (45) has written an extensive article on alterations in the catabolism, disposition, and pharmacological effects of drugs in animal and human embryos.

Techniques in Experimental and Clinical Teratology

The techniques used to produce and demonstrate developmental abnormalities are basic to an interpretation of the teratogenic capabilities of a drug. The species of animal used—even the strain— period of drug administration during gestation, route of administration, dosage, nutritional and endocrine status of the mother, and methods used to examine animals for developmental defects are essential factors. The pregnant rat and mouse

have been most widely used, but important information in interpreting the relevance of the results to man is being gained from other species (46).

The echinoderm embryo (47, 48) has been carefully studied, and it is responsive to a variety of drugs (49). Various drugs have produced specific disturbances in the frog (50) and zebra-fish embryos (51). The chick embryo is readily available and has been intensively studied, morphologically and biochemically (52, 53); and a variety of specific developmental defects can be correlated with the action of certain classes of drugs (54, 55).

The development of skeletal disorders in the mouse has been described in detail (56). Mouse embryos of various strains have shown quantitatively different responses to drugs (57, 58). The site of fetal implantation in the uterus (59) and maternal age (60) are factors in determining the occurrence of congenital abnormalities. The mouse embryo has been used mainly for the exploration of genetic factors in the teratogenic response to drugs, whereas the pregnant rat has been most extensively exploited in screening drugs for teratogenic activity (61). The rabbit fetus, which has acquired new interest since it appears to be the laboratory animal most sensitive to the teratogenic action of thalidomide, has been carefully studied for developmental abnormalities (62, 63). The dog (64) and monkey (65) will undoubtedly become more important in teratology as adequate maintenance facilities become available.

There is no a priori basis for selecting the species most likely to respond to a potential teratogen. For example, the chick, mouse, and rat embryos have been surprisingly resistant to thalidomide, whereas defects have been obtained consistently in the appropriate rabbit strain. The rat fetus is susceptible to the alkylating agents, and the chick embryo is susceptible to antimetabolites. If the mechanism of action of a drug is understood, it should be tested in a responsive species. For most drugs this information is not known, and a variety of species are used empirically.

Separate methods for drug testing must be developed for each species. While individual investigators have worked out their own techniques, attempts are being made to standardize a system which would be acceptable to those concerned with assessing the suitability of drugs for clinical use.

The United States Food and Drug Administration has proposed the so-called "three-litter test" in rats. As summarized by Brent (66):

> The test involves the premating administration of a drug to rats at several dose levels, 60 days before mating. Drug administration continues during the mating period and throughout the gestation period. The animals are allowed to deliver naturally and the offspring are counted, weighed, and examined for abnormalities. The drug is not withdrawn but is given also during the nursing period to determine whether it has any effect on the growth or viability of the young. It is recommended that two additional generations of rats be raised while the parents are receiving the drug.

Brent notes that this test may invoke antifertility mechanisms, including disturbances in libido, maternal nutrition, ovulation, spermatogenesis,

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implantation, endocrine status, lactation, and maternal concern for the young—any of which would complicate the interpretation of the results. Brent has recommended an animal testing protocol; he suggests that the drug be given by the same route as that to be used clinically, if possible, and studied in rats, mice, rabbits, and other species, as suggested by the known pharmacology of the drug. Treatment should be started shortly after fertilization and continued through the late fetal stage. The animals should be obtained at term by caesarean section and examined for defects. Cahen (41), in his excellent analysis of the problem, describes a standardized technique in rats and mice and suggests that the initial study involve the use of a single dose given during the critical period of organogenesis; if the dose induces fetal resorption, then it is reduced to determine the dose-effect relationship. Rather than sacrifice all the fetuses at term, natural delivery should be allowed for some litters; and the young should be followed postnatally for survival and the appearance of abnormalities, including functional, central-nervous-system disturbances. At least two species should be used for each drug. Peck (67) has considered the various types of tests for teratogenesis that have been used, and recommends that "teratogenic studies of new agents should be conducted under reasonable non-toxic daily dosage schedules in the intact mammal . . . "

Lutwak-Mann & Hay (68) administered drugs to rabbits shortly after fertilization and found that certain teratogenic agents, including thalidomide, were toxic to the preimplantation blastocyst. The possibility that certain substances might be teratogenic because of their effect on sperm has been raised (69), and Lutwak-Mann (70) reported deleterious effects on the progeny of 27 of 40 matings of thalidomide-treated male rabbits. This introduces an additional complex factor in the teratogenic effects of drugs (71).

Other factors that must be considered are the potentiating teratogenic effects of combinations of drugs (72, 73) and unusual susceptibilities of the embryo due to the maternal or fetal genotype (74). The latter problem has been discussed in two recent books on pharmacogenetics (75, 76). The possibility that developmental or functional defects may appear postnatally has been studied by Werboff et al. (77, 78) who noted behavioral changes in rats whose mothers received various tranquilizers during gestation. The experimental data for the prenatal shaping of behavior was reviewed recently (79). Grollman & Grollman (80) found that the administration of hypertension promoters, including aldosterone or a high salt or a low potassium diet during pregnancy, resulted in hypertension in the offspring.

Teratogenic studies should be conducted in mammals because of their close relationship to man. The chick embryo is a unique system, however, since drugs are retained after injection into the egg and are, in most cases, slowly metabolized. Certain drugs, even when introduced early in development, do not produce their effects until susceptible embryonic systems appear. Other drugs act promptly when given early in development but lose

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their potency at later stages. The test drug is generally injected into the yolk sac at 4 days of incubation, and embryos succumbing are inspected for developmental defects; those surviving to 18 days are sacrificed for careful study (55). On the basis of the preliminary results, further studies may require changes in dosage, route of administration, and the age of the embryo at the time of injection.

Thus far, no firm bridge has been established between animal experiments and the human situation. Animal data must be obtained, but there is no foolproof or generally acceptable method to ascertain their relevance to man.

The step from laboratory studies to the clinical trial of a new drug must be cautiously undertaken. The unique properties and therapeutic promise of a new drug direct the extent and nature of the clinical evaluation program. There is considerable uncertainty as to how to accumulate data to determine whether a drug will or will not cause teratogenic or feticidal effects in man. It is essential, however, to design clinical methods to identify drugs which can produce disturbances in the human fetus, particularly if the use of the drugs is to be unrestricted. MacBride (81) and Lenz (82) independently observed an association between the use of thalidomide by the mother and an increased incidence of specific birth defects without the help of systematic survey facilities. It is hoped, however, to accelerate such recognition of environmental teratogens by organized detection methods. This requires, first, the establishment of a birth-defect-reporting registry. This has been done, for example, in New York State (83). Monthly reports during a 10-month period (May, 1962 to March, 1963) showed a malformation rate ranging from 1.021 to 1.287 percent.

There are many factors involved in analyzing the incidence of fetal deaths and birth defects. A general orientation in the problem, based on a review of the literature (84, 85) and discussion (86), may be made. The area of concern is the mortality and morbidity of the fetus, beginning at the time of conception and extending to the end of the first postnatal year. It is estimated in the general population that there is an approximately 20 percent periconceptional loss, i.e., pregnancies which do not terminate in a live birth. This figure cannot be accurately established because of the difficulty in proving early pregnancy. In Mellin's fetal life table, 6.8 percent of fetal deaths occurred between the seventh week of gestation and the completion of pregnancy; this figure is somewhat lower than other series, where the fetal deaths may be as high as 10 percent. The condition of these dead fetuses was studied, and a total of 16.8 percent was malformed; 9.3 percent were diagnosed clinically and 7.5 percent were examined by autopsy. Carr (87) observed that 8 out of 35 specimens obtained from spontaneous abortions, mostly during the first trimester, showed major chromosomal abnormalities. In live births, and this includes babies that have survived for at least 28 days after birth, the incidence of major defects is in the range of 2 percent. In a detailed series reported by Marden et al. (88), they observed

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a major malformation rate in live births of 2.04 percent and a minor malformation rate of 14.7 percent.

The major abnormalities were random, involving the central nervous system, craniofacial area, oral and digestive tract, eye, ear, skin, extremities, musculoskeletal system, and genitourinary tract. It has been noted, also, that occult malformations may be present at birth but may not become manifest until during the first year of life. The incidence of postnatal malformations may be equal in number to those found during the neonatal period. In Japan, the incidence of external malformations at birth is 1.8 percent, and it is noted that only 42 percent of infants with major malformations survive to leave the hospital (89). Carter (90) comments that while birth defects range from 2 to 3 percent throughout the world, there are racial differences:

> Examples of such variation are: the high incidence of anencephaly and spina bifida cystica in northwest Europeans, especially in Ireland, part of Scotland and South Wales; the high incidence of cleft of the lip and palate in Japanese; the high incidence of preauricular fistula and polydactyly in Negroes; the high incidence of dislocation of the hip in Lapps and certain American Indians.

The causes of these developmental abnormalities are largely unknown. The major factors are genotypic determination, defective sperm (91) or ova, and intra-uterine disturbances; radiation and certain virus infections are exogenous contributing factors of lesser importance.

There is little evidence, with the exceptions noted earlier, that drugs have a responsible role in the great majority of fetal deaths and birth defects. In a recent study, Mellin (92) compared the incidence of fetal deaths and birth defects in a group of mothers who took one or more prescribed drugs during pregnancy and in a control group of similar size who received no medication. The fetal loss was slightly higher in the group receiving drugs, possibly because of the need for drugs in the more complicated pregnancies. There was no significant difference, however, in the malformation rates of the two groups.

Recent statistical studies have not detected an increase in the incidence of malformed infants born into the population. A report of an increase in the incidence of meningocoele in Atlanta, Georgia (93), could not be supported on statistical grounds (94). Lucey et al. (95) also noted an increase in the incidence of spina bifida in Vermont; the significance of this observation remains to be determined, but they do not believe it was by chance. In a statistical study on thalidomide malformations (96), it was found that the incidence of hypoplastic and aplastic anomalies of the extremities and ears occurring in West Germany ranged, during 1950 to 1959, between 0.3 and 0.7 per 1000 of live births. Subsequently, the rate rose sharply: in 1961, it was 3.07 per 1000, and the first half of 1962 showed 2.5 per 1000. Thalidomide was withdrawn from the market in late 1961; and, by the latter half of 1962, when the effects of withdrawal of thalidomide became demonstrable,

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the rate dropped back to 0.62 per 1000. Furthermore, the birth defects seen during 1961 and early 1962, and, interpreted as characteristic of thalidomide, did not resemble those seen during late 1962. When a highly active and widely used teratogen appeared and was then removed from the environment, its effect could be readily recognized by these recording methods.

It is hoped that population surveillance will reveal unexpected teratogenic influences. The unprecedented thalidomide disaster, however, does not seem likely to be repeated. While pharmacologists are working with highly specific and potent drugs, knowledge of their mechanism of action will often indicate those with teratogenic potentialities. Preclinical drug trials in pregnant animals, conducted in an organized manner, will cast further light on the teratogenic activity of a drug. Extensive trials of a new drug will not be conducted during pregnancy unless it has a therapeutic or life-saving advantage in specific situations. There is a vigorous trend toward the elimination of the unessential use of drugs during pregnancy. Prospective statistics are being gathered, reaffirming, in most cases, the safety of widely used and established therapeutic agents which may be indicated for use during pregnancy. It is necessary to be constantly on the alert, however, for an unexpected teratogen in the environment; and the seriousness of the problem cannot be minimized. One must agree, nevertheless, with Lutwak-Mann's (97) statement:

> It seems that the capacity of the embryo to withstand certain injuries is at least as impressive and fundamental as the previously discussed sensitivity to extraneous agents.

Drugs of Current Interest

The teratogenic drugs in animals and man have been listed in many papers, including the 1964 review in this series (98). While reports of new teratogenic agents in animals are accumulating, the chemical teratogens in man are now well known (99–103).

Anti-cancer drugs.—Drugs which interfere with the growth of proliferating tissues, whether normal or neoplastic, may be expected to cause developmental abnormalities (104). These drugs are known either to block biosynthetic processes necessary for cellular replication, to act directly on DNA, or to inhibit cell division (105). In the treatment of neoplastic disease, anti-cancer drugs are generally given in the range of maximum tolerated dose, which further increases their teratogenic potential in man. Sokal & Lessmann (106) summarized the clinical reports on the effects of anti-cancer drugs administered during pregnancy. Despite the presumed risk, they conclude that with the exception of the folic acid antagonists "other drugs in clinical use have proved far less toxic for the fetus than would have been anticipated from theoretical considerations or animal experiments."

Polyfunctional alkylating agents.—Representative polyfunctional alkylating agents caused a wide range of teratogenic effects in the pregnant rat,

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when given intraperitoneally on the eleventh to twelfth days of gestation (107). The compounds tested included nitrogen mustard, chlorambucil, cyclophosphamide, triethylene melamine, ThioTEPA, and busulfan. All the drugs produced retardation and abnormalities of the extremities and tail, but qualitative differences appeared in the incidence of cleft palate and encephaloceles. Monie (108) found that unilateral and bilateral absence of the kidney was a frequently encountered abnormalities in the offspring of pregnant rats treated with chlorambucil on the tenth day of gestation. A 4.5-month-old fetus, obtained from the termination of pregnancy in a 27-year-old patient with Hodgkin's disease who was receiving chlorambucil, had an absent left kidney and ureter (109). By external inspection, the fetus appeared to be normal. An abnormal fetus was delivered from a 22-year-old patient with Hodgkin's disease who had been treated with cyclophosphamide intravenously for a 6-day period. During the estimated seventh to eighth week of pregnancy (110), the total intravenous dose was 27.5 mg per kg, and oral cyclophosphamide therapy preceded and followed the intravenous course. The infant was a male of premature weight, with hand and foot defects, bilateral inguinal hernial sacs, and grooves extending to the uvula on each side of the midline of the hard palate. The infant has survived and has a normal karyotype. There is strong presumptive evidence that the polyfunctional alkylating agents are capable of producing developmental defects in man.

Anti-metabolites.—An increasing number of highly potent anti-metabolites of interest in cancer chemotherapy have produced fetal resorption or teratogenic effects in laboratory animals (41). In a classical study by Thiersch (111), aminopterin, administered to 12 women during the third to eighth wk of gestation caused spontaneous delivery within 5 to 17 days in ten patients; and, in two, the uterus was surgically evacuated. The latter two fetuses showed developmental defects. The teratogenic effect of the folic acid antagonists has been confirmed (112). Purine analogues (113) related to 6-mercaptopurine (61) have produced fetal death and severe stunting in the surviving embryos in the rat. The xanthine oxidase inhibitor, 4-hydroxypyrazolopyrimidine, which inhibits the oxidation of 6-mercaptopurine to thiouric acid, enhanced the teratogenic activity of 6-mercaptopurine about fourfold in the rat (114). Hadacidine (N-formylhydroxyaminoacetic acid) (115), an antitumor antibiotic which acts by inhibiting adenine biosynthesis, produced severe developmental abnormalities in the fetus of the Wistar rat. The high incidence of cleft palates produced by hadacidin has made the drug useful in studying the evolution of this defect (116). The glutamine antagonists, 6-diazo-5-oxonorleucine (DON) and *o*-diazoacetyl-1-serine (Azaserine), which also interfered with purine biosynthesis, produce fetal deaths and abnormalities at doses far below those toxic to the mother (61).

The fluorinated pyrimidines, including 5-fluorouracil, cause defective development in a high percentage of rat and mouse fetuses (61, 117).

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Murphy (61) noted that FCdR (5-fluorodeoxycytidine), while less toxic than FUdR (5-fluorodeoxyuridine) to the mother rat, was about 50 to 100 times as effective by weight, when administered to the pregnant rat, in causing fetal resorption or developmental defects. This may be explained if the fetus lacked a deaminase which converted FCdR to FUdR and thereby hastened its breakdown to a less active form. The results illustrated, perhaps, the importance of metabolic differences between the mother and fetus at specific stages of development.

The substitution of other halogens (Cl, Br, I) in place of fluorine in FUdR produces drugs whose mechanism of action differs from FUdR. IUdR has produced defects of the hind limb in the offspring of mice treated with 5-BUdR (118); and the Cl, Br, and I analogues of FUdR are also teratogenic in rats (61). The teratogenic effects of ClUdR in rats can be prevented by the administration of thymidine (119). Cytosine arabinoside (120), an antimetabolite which appears to act by interfering with the reduction of cytidylic to deoxycytidylic acid, induced consistent abnormalities and severe growth inhibition in the chick embryo, defects which could be prevented by the administration of deoxycytidine.

Anti-tumor antibiotics.—The toxic antibiotics, which have been considered as anti-cancer drugs, are generally teratogenic. Actinomycin D has been active in the chick embryo (121, 122) and rat embryo (123), particularly when given early in development. Actinomycin D was also postulated to interfere with blastocyst and endometrial development in the rat (124). Takaya (125) found, in hybrid albino rats, that actinomycin C, mitomycin C, sarcomycin, carzinophilin, and chromomycin A-3, when injected subcutaneously on the sixth to tenth days of pregnancy, produced malformations. Actinomycin C and mitomycin C produced the largest percentage of abnormal fetuses. Streptonigrin (126), when injected intraperitoneally into the pregnant rat on the ninth and tenth day of gestation, produced malformations in the spine and spinal cord as well as in other organs.

Metaphase inhibitors.—The plant alkaloids, colchicine, podophyllotoxin, vinblastine, and vincristine, have all shown teratogenic activity in animals. Ferm (127) injected colchicine intravenously in the pregnant hamster on the eighth day of gestation and obtained high fetal morbidity, and the survivors showed exencephaly and skeletal abnormalities. Eye defects were also observed with vincristine and vinblastine, but colchicine was the most active agent (128, 129). In rats, vinblastine produced a high incidence of fetal resorption (130); and 24 of 308 young at term (8 percent) showed gross abnormalities (131). Podophyllotoxin and podophyllin caused resorption of the fetal rat (132). A recent clinical report, describes a 24-year-old woman who took "slimming tablets" containing podophyllium during the fifth to ninth weeks of pregnancy and gave birth to a baby with multiple deformities (133). Armstrong (134) reported a patient who became pregnant and delivered a normal baby while on oral vinblastine; and Lacher (135) treated a patient with intravenous vinblastine, a total of 47.8 mg, beginning in the fifth month of gestation, who then delivered a normal infant. Whether

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an equally happy result would have occurred if the patient had been treated during the first trimester is not known.

Miscellaneous drugs.—N-methylhydrazine [N-isopropyl-*O* (2-methylhydrazino)-*p*-toluamide hydrochloride] (136) and hydroxyurea (137), two drugs of recent interest in cancer chemotherapy, are both active teratogens in rats.

Steroid hormones.—These substances which act, in various ways, to inhibit or stimulate the growth of specific tissues, can be teratogenic in animals and man. The adrenocortical steroids produce a high incidence of cleft palate in the fetuses of pregnant mice of appropriate strains, and this phenomenon has been intensively studied by Nishamura (17). Similar effects have been observed when the rat fetus was directly injected with cortisone (138) and in pregnant rabbits treated with cortisone (139). More extensive developmental disturbances were observed in rabbits following Soludecadron (140). Watney & Miller (141) made a remarkable observation on the offspring of C3H/M1 mice, which have a 70 percent incidence of a congenital abnormality, known as "open eye" at birth. The administration of cortisone, at a period of development just prior to the normal fusion of the lids, prevented the appearance of this defect. By careful adjustment of dosage, the "open-eye" defect could be prevented without producing cleft palate and a high postnatal mortality. It is suggested that cortisone prevented the appearance of a genetic defect by influencing connective tissue formation and thus decreasing abnormal skin tension in the region of the eyes in this strain of mouse.

There is some evidence that adrenal steroid therapy during pregnancy in man may cause fetal abnormalities; Bongiovanni & McPadden (142), in a survey of the world literature up to 1960, reported cleft palates in two of 260 pregnancies during which adrenal steroids were administered; they also refer to two other patients observed by Grumbach. In their group, there were eight stillborn, one abortion, fifteen premature and seven full-term infants with various disorders. They conclude that "the human fetus is rarely injured by maternal treatment with corticoids." Popert (143) discussed the use of adrenal steroids or corticotropin in 21 pregnancies: four terminated in abortion, one in fetal death, and one baby had a cleft palate; but there is no evidence that these mishaps were related to the steroid therapy. One case is reported (144), however, of a patient who received large doses of prednisone for sarcoidosis for 24 days prior to the spontaneous delivery of a six-month-old fetus which died in 3.5 hr. The adrenals were small and cystic, and it is suggested that the exogenous steroids suppressed the development of the fetal adrenals, and the fetus was suffering from acute adrenal insufficiency.

While progesterone and 17-hydroxyprogesterone caproate are not teratogenic (145), certain derivatives can cause virilizing effects in animals and in man. Wilkins (146) reported that 17-α-ethinyl-testosterone and, to a greater extent, 17-α-ethinyl-19-nor-testosterone used during pregnancy caused virilization in female offspring. In a survey by Bongiovanni &

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McPadden (142) of 650 pregnant woman receiving 17-ethinyl-testosterone or its 19-nor derivative, virilization of the fetus was observed in only two cases. Jacobson (147) reported, however, that 15 of 82 female infants (18.3 percent) born of mothers receiving 17-α-ethinyl-19-nor-testosterone showed evidence of masculinization. It is noted that there is no progressive evidence of virilization after birth, and the deformity is relatively easy to correct. On the basis of their experience in 100 cases, Hagler et al. (148) suggest that it is relatively safe to administer steroids during early pregnancy, if indicated, in small doses. They note that 19 genetic male babies whose mothers received estrogens during pregnancy did not show pseudohermaphrodism or feminization.

There is no doubt that androgens, given during pregnancy, can be virilizing to female fetuses. This has also been reported following diethylstilbestrol (149), but is apparently a rare complication.

The steroid hormones, while potential teratogens, are to be used with caution and only when indicated but nevertheless appear to be surprisingly limited in their effects on the fetus.

Insulin and hypoglycemic agents.—Agents which decrease the blood sugar in mice can produce fetal death and developmental defects in the survivors (150).

In the chick embryo, insulin was found to produce developmental defects consisting of rumplessness and shortening of the extremities, and these were dependent on the age of the embryo at the time of injection. The histological evolution of this disturbance (151) and related biochemical changes (152) have been studied by Sevastikoglov. Insulin has also caused abnormalities in the mouse and rabbit fetus (41).

The sulfonylurea derivatives, used in the treatment of diabetes, are teratogenic agents in laboratory animals. Tolbutamide produced abnormalities involving the heart, eye, and extra- and intra-embryonic circulation of the Medaka fish, Oryzias latipes (153). Lazarus & Volk (154), however, did not observe any abnormalities in the offspring of pregnant rabbits receiving tolbutamide from the seventh to fourteenth day of gestation. Tolbutamide has been suggested as a possible cause of multiple developmental defects in a 7½-month-old fetus from a diabetic mother who received this drug continuously during pregnancy (155); however, diabetic mothers have a higher incidence of abnormal fetuses (156) than normal mothers, and tolbutamide may not be the teratogenic agent in this case. Chlorpropamide is possibly teratogenic in man (157), and carbutamide is teratogenic in laboratory rodents (158). On the basis of clinical experience with oral antidiabetic drugs, Sterne & Lavieuville (159) stated that there is no firm support for the suggestion that the various sulfonylurea derivatives or dimethylbisguanidine are significant as teratogens in man; Malins et al. (160) reached a similar conclusion.

Alloxan, which causes diabetes by damaging the insulin-secreting cells of the pancreas, produces developmental abnormalities in the fetuses of

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treated mice (161); these effects were prevented by insulin (162). The use of insulin or sulfonylurea derivatives, when indicated as antidiabetic agents, does not appear to cause a significant increase in developmental defects in the human fetus.

Vitamins: excesses, deficiencies, and antagonists.—Abnormalities in vitamin levels in either direction or specific vitamin antagonists produce teratogenic effects in animals. With the exception of the folic acid antagonists (111), the relevance of these laboratory observations to man is uncertain. The first report, in 1933, of induced developmental defects in a mammal is attributed to Hale (163). As a result of a vitamin-A deficiency, piglets were born without eyeballs. This teratogenic effect of a vitamin-A deficiency was confirmed in other laboratory animals, but of greater interest was the finding that vitamin A, in excessive dosage, also produced consistent defects in the mouse and rat fetus. The oral administration of vitamin A to normal and hairless mice produced consistent fetal abnormalities—chiefly, cleft palate and microphthalmia; the abnormalities did not exceed control levels if the vitamin A were given parenterally in corn oil (164). Extensive abnormalities were produced in several strains of mice by the administration of vitamin A through a stomach tube (165). Anomalies of practically every organ system were observed, some of which appeared to be related to defects found in human beings. Vitamin A by mouth or by injection in pregnant rats produced a high incidence of fetal abnormalities which could not be influenced by cortisone, insulin, or growth hormone (166).

Riboflavin deficiency in pregnant rats causes skeletal abnormalities and cleft palate, and these abnormalities are increased by the simultaneous administration of the riboflavin antagonist, galactoflavin. It is suggested that the developmental defects are caused by an observed decrease in the flavin adenine dinucleotide levels in the embryo (167).

A nicotinamide-deficient diet, together with 6-aminonicotinamide, a nicotinamide antagonist, produced a high incidence of fetal resorption in the rat and developmental abnormalities in the surviving fetuses (168). Similar results are obtained with a single injection of 6-aminonicotinamide, and developmental defects can be prevented by the simultaneous or prior administration of nicotinamide (169). Ingalls et al. (170, 171) have observed, in mice, polyploidy and fragmented chromosomes in cells of both the bone marrow of the mother treated with 6-aminonicotinamide and her abnormal fetus. The possibility is raised that one may produce hereditary anomalies by this technique. Strain differences have been found in the abnormalities produced by brief deficiencies induced by 6-aminonicotinamide in the mouse in relation to the incidence of vertebral fusion and cleft palate (172). The hereditary factors influencing susceptibility to the teratogen appear to differ for the respective organ analogues. Two nicotinamide antagonists, 3-acetylpyridine (3-AP) and 6-aminonicotinamide (6-AN), produce distinct developmental abnormalities in the chick embryo; the former causes muscular hypoplasia, and the latter causes dwarfing, micromelia, and

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parrot beak. While nicotinamide will protect against both antimetabolites, 3-AP, at nontoxic doses, will prevent the teratogenic effects of 6-AN; several explanations for this effect are discussed by Landauer & Clark (173). A flavinoid derivative (4′,5,6,7,8-pentamethyloxyflavone) was highly toxic to zebra fish (174), and this drug in pregnant rats resulted in young who were not viable or died shortly after birth (175).

Antibiotics and drugs used against infectious organisms.—These drugs are used, for specific indications, during pregnancy. Some of the agents in this group have produced teratogenic effects in laboratory animals. The antiviral drugs, 5-iododeoxyuridine and cytosine arabinoside, which are used locally in infections against DNA-containing viruses, are potential teratogens in man as noted earlier.

It has been reported that streptomycin, when administered during pregnancy, was related to impaired hearing of varying severity in three of ten children (176). In C_{57}BL mice, streptomycin readily passed the placental barrier; no developmental malformations were seen grossly, but 9 of 52 embryos showed slight microscopic abnormalities on histological study of the brain (177). The significance of these observations is not known. In rats and in women treated with dihydrostreptomycin or kanamycin during pregnancy, there was no evidence of gross defects or impaired auditory function (178). In a clinical study on patients with tuberculosis who received isoniazid and streptomycin, no increase was found in fetal deaths or congenital malformations as compared to a control group (179).

When tetracycline is injected into the yolk sac of the chick embryo, it is localized in the skeletal system. At high doses, there is a decrease in the size of the embryo; and the bones are small, bowed, and incompletely calcified (180). Inconclusive clinical data raised the possibility that the administration of tetracycline to the mother may have a role in the appearance of congenital cataracts (181). A 900 g nonviable human fetus, whose mother had taken 1 g of tetracycline daily for three weeks prior to the birth of the child, had considerable tetracycline fluorescence in a considerable portion of the mineralized regions of the skeleton (182). As a consequence, the adverse effects of tetracycline were studied in postnatal premature infants. Bone growth was inhibited during the period of tetracycline administration, but it recovered promptly when the antibiotic was stopped. Transplacentally acquired tetracycline has been shown to stain the deciduous teeth of the infant (183).

Quinacrine was administered during the first trimester of pregnancy for the treatment of polyarthritis; the term fetus died with jaundice, agenesis of the kidney, right-sided hydronephresis and spina bifida (184).

Cohlan (103) has concluded that "there is little direct evidence of fetal damage from the antibiotics which freely transverse the human placenta."

Sedatives, tranquilizers, and analgesics.—These drugs are widely used and are often casually prescribed. The unprecedented teratogenic potency of thalidomide in man directed a high level of suspicion toward other members

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of this therapeutic group and forced their careful consideration for evidence of teratogenic activity.

The thalidomide story has been told many times (98, 185). In late 1961, this relatively nontoxic sedative was shown to be responsible for characteristic deformities in a large number of fetuses from mothers who had received the drug. Careful analyses showed that the critical period of drug administration as far as teratogenic effects were concerned was from the fourth to seventh week of pregnancy, and a single dose of as little as 50 mg (about 1 mg per kg) could result in a deformed baby (16). While some thalidomide babies die early or are aborted, a large number survive with serious morphological defects. The described abnormalities, which are in part related to the stage of embryonic development when thalidomide is administered, included the following types: (*a*) extremities: amelia, phocomelia, and hemimelia; (*b*) skeleton: rudimentary scapulae and maldevelopment of sacrum; (*c*) external lesions: absence or dysplasia of ears and eyes, hemangioma, nose and upper lip, and cleft palate; (*d*) digestive tract: atresia of esophagus, duodenum, anus, and aplasia of gall bladder and appendix; (*e*) miscellaneous: cardiac anomalies, hydrocephalus, renal agenesis, and genitourinary abnormalities.

The incidence of abnormal offspring from patients who took sedative doses of thalidomide during the critical period of gestation is uncertain, but one estimates it is about 20 percent (186); and it is possibly even higher. It may be presumed that a higher incidence of birth defects would occur with larger doses of thalidomide, indicating that the drug has a consistent teratogenic action in man.

The effect of thalidomide could not have been predicted: it did not appear to be growth-inhibiting nor to produce appreciable metabolic changes in the adult patient; and it was used clinically in relatively small doses, far below those causing acute toxicity in man and in animals. It is thus mandatory to determine how thalidomide acts on the human fetus.

It was first necessary to find a suitable laboratory animal in which to study the teratogenic effects of thalidomide (41). There are quantitative disagreements among the results obtained in some species, but a pattern of the toxicity of thalidomide for mammalian embryos is emerging.

Somers' (187) original report on the teratogenic effect of thalidomide in the New Zealand rabbit has been amply confirmed (188, 189, 190). Whereas he administered a dose of 150 mg per kg from the eight to sixteenth days of gestation, Felisati (191) obtained consistent, but less severe, abnormalities with an oral dose of 50 mg per kg from the seventh to the fifteenth days of gestation. Sawin (192) used a daily oral dose of 500 mg per kg from the sixth to eleventh day postcopulation in four strains of rabbits. There were 91 viable offspring; only four appeared normally formed, and the remainder had malformations involving the major organ systems, with the type of defects modified to some extent by the genotype of the fetus. The critical period for producing malformations appears to be during the eighth to

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eleventh days of gestation; if thalidomide administration were begun after the tenth day, no malformations were observed. Also four rabbits who gave birth to malformed young were remated, and none of the offspring was abnormal (193). Thalidomide affected the rabbit blastocyst (68, 193), but apparently recovery can occur; the highest percentage of fetal abnormalities surviving to term occurred in rabbits receiving the drug during the eighth to tenth days of pregnancy. Seller (194) found that fetal abnormalities occurred in New Zealand rabbits receiving 50 mg per kg of thalidomide intraperitoneally daily from the first to the twelfth day of gestation, whereas a Silver Gray strain was not affected.

The rat fetus is not highly susceptible to the teratogenic action of thalidomide (41, 66, 195). Klein Obbink & Dalderup (196) have described a decrease in litter size and the presence of skeletal defects in fetuses obtained from rats who received up to 2 g per kg of thalidomide daily from the sixth to twenty-first day of gestation. Similar results were reported by McColl, Globus & Robinson (197). In an extensive study in the rat, Kopf, Lorenz & Salewski (198) found that thalidomide intefered with the early zygote and increased uterine resorptions, but malformations did not occur. Thalidomide-induced abnormalities in the mouse fetus have been irregular, and the incidence has been low (41). There is an increase in fetal resorption; and the young are stunted at birth, although malformations are not evident (199). Di Paoli (200) observed embryos with congenital abnormalities in seven of 62 litters of strain-A mice whose mothers received 31 or 62 mg per kg of thalidomide by intubation for 4 to 5 days, starting between the second and tenth days after conception. Higher doses early in pregnancy caused a failure of implantation in 10 of 22 litters. Giroud, Tuchmann-Duplessis & Mercier-Parat (201) noted an incidence of malformations in Swiss A/HC and Swiss Albino mice; whereas Knoche & Konig (202) in NMRI mice noted stunting in the survivors and a higher level of failure of implantation, presumably due to blastocyst destruction; but only two of 369 fetuses surviving to term were defective.

In the monkey, 50 to 200 mg daily of thalidomide by mouth for, usually, 33 to 45 days early in pregnancy prevented live births, presumably because of destruction of the embryo prior to implantation (203). Weidman et al. (204) obtained skeletal defects in surviving puppies whose mothers received 100 mg per kg for one to 21 days postconception. Hamsters (205) receiving approximately 350 mg per kg of thalidomide by mouth for 15 days postconception showed a suggestive, but slight, increase in fetal resorptions and developmental abnormalities.

The effects of thalidomide on the chick embryo have not been consistent (41). The percentage of abnormal embryos surviving to the time of hatching has not been high (206), ranging from 20 to 30 percent; and hydroxy derivatives of thalidomide and glutamic acid analogues appeared to be equally active (207). Williamson (208) observed that the injection of colloidal material into the yolk sac of the chick embryo caused developmental defects which

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were similar to those seen with thalidomide; our own experience has been similar, in that 2, 2.5, and 20 mg of thalidomide injected at 0, 2, and 4 days, respectively, into the yolk sac failed to produce a significant number of abnormalities in embryos surviving to 18 days (209). Feeding hens with thalidomide did not interfere with egg laying; but there was an increase in the number of nonfertile eggs, fertile eggs with no development, or early death of embryos; there was no increase in the incidence of gross malformations (210). Thalidomide appears to be toxic to the preimplanted and implanted blastocyst of the armadillo (211).

From the biological data on several mammalian species, the blastocyst appears to be sensitive to thalidomide administered early in development. When administered during the period of implantation and early organogenesis, the most consistent teratogenic effects are obtained in the rabbit; the mouse, rat, hamster, and chick embryo appear to be less satisfactory for teratological studies.

The mechanism whereby thalidomide damages the fetus has not been established. It is possible, particularly because the human embryo is so much more sensitive than those of other species, that thalidomide is converted by some species to a more active derivative *in vivo*. The metabolism of thalidomide has been considered in the preceding volume of the *Annual Review of Pharmacology* (212). Misiti et al. (213) found that *dl*-3-phthalimidoglutaramide and N-phthaloyl-*dl*-aspartimide were teratogenic in the chick embryo and increased the incidence of fetal resorptions in the rat. Using New Zealand rabbits as an assay system, Wuest, Sigg & Fratta (214) obtained abnormal fetuses with 2-phthalimido-N-methyl glutarimide, whereas none occurred with 2-[hexahydrophthalimido]glutarimide. They concluded that the aromatic ring is essential for teratogenic action, whereas the replacement of the imido hydrogen by a methyl group to yield methylated glutamine or isoglutamine on hydrolysis did not alter teratogenic activity. Soluble hydroxy derivatives of thalidomide, *d*-(N-3-OH-phthalimido)-glutarimide, and 3-OH-phthaloylglutamine inhibited the growth of mouse embryo cells in tissue culture at concentrations of 0.3 mg per ml; the effect of thalidomide was inconclusive (215). Roath, Elves & Israels (216), using short-term cultures of human leucocytes containing phytohemagglutinin, demonstrated that thalidomide and some of its degradation products inhibited lymphocyte proliferation. The significance of this study is questioned by Lindahl-Kiessling & Böök (217), who found that thalidomide did not show any antimitotic activity in 27 of 30 leucocyte cultures, Ehrlich ascites cells, exposed in a medium containing 250 μg per ml of thalidomide, produced an increase in mitotic activity which could not be prevented by nicotinic acid, folic acid, pyridoxal, or riboflavin. Oxygen uptake of the tumor cells was unaffected (218). Thalidomide and its derivatives thus appear to be inert or only weakly active in inhibiting cell growth or the metabolic processes studied; the circumstances resulting in marked susceptibility to thalidomide may be operational, however, in embryos of certain species at a critical stage in development.

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Meclizine and cyclizine have been used to treat the nausea of pregnancy. A preliminary epidemiological report in England in December, 1962, suggested that the use of meclizine was associated with ten congenital malformations (219). King (220) found that high doses of meclizine in rats on the twelfth to the fifteenth days of gestation produced severe malformations of the head, vertebrae, and limbs. This was associated with a transient increase in the volume of amniotic fluid (221). Teratogenic effects were observed, also, in fetuses of laboratory rodents receiving cyclizine (222). While Pettersson (223) reported 31 malformed infants in a newborn population of 585 (incidence of 5.3 percent), whose mothers received meclizine as compared to a 2.6 percent malformation in those not receiving the drug, many other studies have shown no increase in birth defects following meclizine or cyclizine (224–228).

Phenothiazine and other derivatives.—Imipramine, in large doses, during the first 13 days of gestation was teratogenic to the rabbit (229). Trifluoperazine (230) and promethazine and pipamazine (228) did not appear to be teratogenic in man. Methaqualone hydrochloride, a hypnotic, produced fetal resorption and developmental abnormalities at doses which also cause severe maternal toxicity in the rat and rabbit. These results are not regarded as evidence of specific teratogenic activity and do not suggest that methaqualone would be hazardous in man (231).

Unrelated drugs in laboratory animals.—Warkany & Takacs found many developmental abnormalities including central-nervous-system lesions, facial defects, and eye defects in the offspring of rats treated with methyl salicylate during the ninth to eleventh days of gestation (232). The mechanism of salicylate teratogenesis is not known; but it was found that an acidic salt load potentiated, while a basic salt load antagonized the teratogenic action of sodium salicylate (233). Sodium salicylate, in a single intramuscular dose of 10 mg to the pregnant mouse during the seventh to thirteenth day of gestation caused severe disturbances in skeletal and vascular development (234); and aspirin increased the incidence of stillbirths in rats and mice (235). Since dinitrophenol is not teratogenic in rats and mice, the salicylates do not exert their effect on the fetus by uncoupling oxidation and phosphorylation (233, 236).

Serotonin produced fetal abnormalities, including defects of eyes, limbs, tail, skull, and central nervous system in pregnant mice receiving 5-OH tryptamine on the fifth to twelfth days of gestation (237). These effects are thought to be mediated through a direct action on the placenta (238). Reddy, Adams & Baird (239) reported that serotonin caused an increase in fetal resorptions, macerated fetuses, and developmental abnormalities in the rat. One patient who had a functional carcinoid tumor, which causes an increase in serotonin production, during four pregnancies had three infants who died within 2 to 36 hr after birth with respiratory difficulty and cyanosis; and the fourth child had multiple malformations. In a second patient, pregnancy resulted in an abortion during the fourth month of gestation.

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The oral administration of triparanol, which caused protracted hypocholesterolemia, resulted in a high number of fetal deaths in the Wistar rat; malformations including facial, skeletal, and ocular defects were more common in fetuses whose mothers were treated on the fourth day of gestation (240). The impaired conception, increased fetal resorption, and birth defects caused by triparanol could be corrected by ACTH (241).

Trypan blue and several related compounds (242–244) and Congo red (245) are effective teratogens in rats. Bins et al. (246, 247) have described a cyclopean-type malformation in lambs whose mothers grazed on *Veratrum californicum* during pregnancy.

Conclusion

A review of drugs as teratogenic agents is a complicated and frustrating assignment. Practical considerations are involved in the need to identify the drugs which may interfere with the development of the human fetus. Basic research is essential to determine how drugs can disturb normal development.

The proved teratogens for the human fetus include the growth-inhibiting anti-cancer drugs, of which the folic acid antagonists have been most carefully studied, the steroid hormones, and the sedative, thalidomide. The anticancer drug category is particularly hazardous, for, among other reasons, these drugs are used therapeutically at near-toxic doses. The known biological actions of the steroid hormones explain their effects. Thalidomide, however, remains an enigma, and is a discordant note in any attempt to predict which drugs may exhibit teratogenic activity in man. While a number of other drugs are suspected teratogens for the human fetus, none have been substantially implicated; in fact the inconsistencies in the response of the fetus to proved teratogens greatly increase the difficulties of interpreting the more tenuous relationships suggested in isolated reports of malformations. The apparent inconsistency in drug effects on the human fetus may be due, in part, to the fact that most drugs have a wide therapeutic index; and patients usually are treated in the dose range adequate to produce the desired beneficial effect. There are critical embryonic periods, such as implantation and organogenesis during the first trimester; and the drug must be present in relation to these specific events to result in a significant effect, by causing either fetal destruction or developmental defects if the embryo survives. Drugs with presumed teratogenic activity can be studied in patients on whom a therapeutic abortion is planned; if this type of study were more generally feasible, many practical problems could be solved. On the other hand, drugs of great therapeutic importance and deemed, from all available evidence, to be safe for the fetus could be examined in an appropriately designed large-scale prospective study. Meanwhile, the practical effects of the thalidomide episode have been to reduce the use of drugs in pregnant women, to look for evidence of drug exposure during pregnancy in the case of any malformed fetus, and to monitor the occurrence of malformations in the population.

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The results of studies on laboratory animals are of uncertain relevance to man. Each species has its characteristic placental functions, maternal endocrine status, embryonic biochemical differentiation, and organogenesis, etc. and operates on its own time schedule in terms of changing interrelationships of these different factors and the speed with which the processes of embryogenesis are completed. Superimposed on this complex and changing system is the introduction of the drug itself, at varying doses which are often greater on a body-weight basis than the therapeutic dose recommended in man, the frequency of administration and different stages of gestation, and the modification of its effects by its disposition in the body, its placental transfer, and its duration of action. While the significance of the animal data in relation to man is often difficult to interpret, testing drugs for teratogenic action in pregnant laboratory animals will be an integral part of the preclinical pharmocological studies.

Progress in understanding the teratogenic effects of a drug will come from a systematic exploration of all of the involved factors, as noted above, in several representative species. The mechanism of action of a drug on a subcellular, cellular, and organ level will be related to detailed information on maternal physiology, placental function, and embryological development for each species. If this is done for several important drugs, model systems can be constructed from which the factors involved in producing fetal abnormalities can be interpreted and weighed. This is a major responsibility of pharmacology; but, thus far, it has been met only in a fragmentary and random manner.

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