REVIEW ARTICLE

Teratology on the crossroads: historical aspects and modern approaches

Eduard Ujházy, Mojmír Mach, Jana Navarová, Michal Dubovický

Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Bratislava, Slovak Republic

Correspondence to: Assoc. Prof. RNDr. Eduard Ujházy, PhD.
Laboratory of Developmental and Behavioural Toxicology,
Institute of Experimental Pharmacology and Toxicology,
Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovak Republic.
tel: +421 2 59410 664; fax: +421 2 5477 5928; e-mail: exfaujha@savba.sk

Submitted: 2012-05-15 Accepted: 2012-05-25 Published online: 2012-05-27

Key words: **history; principles of teratology; congenital defects; functional and neurobehavioural teratology**

Neuroendocrinol Lett 2012; **33**(3):304–313 **PMID:** 22635090 NEL330312R01

Abstract

Teratology is the science of congenital developmental disorders (CDDs), overt or latent defects of the organism resulting from the effect of internal and external factors on developmental processes. In this article the significance and position of present-day teratology is discussed in the context of development of this branch of science and related disciplines. The authors present an updated overview of the most important milestones and stages of the development of teratology. Based on the analysis of the historical development of theses and theories that represent a decisive contribution to this field, we present a survey of the fundamental principles of experimental and clinical teratology. The aim of observing these principles is to get insight into developmental relations and to understand mechanisms of action on the level of cell populations (elementary morphogenetic processes), tissues and organs. It is important to realize that any negative intervention into the normal course of these processes, either on genetic or non-genetic basis, inevitably leads to a sequence of subsequent changes resulting in the development of congenital developmental disorders.

Despite modern approaches of molecular biology and genetics, along with top diagnostic techniques, we are still not able to identify the actual cause in more than 50% of all congenital defects. One-half of the unidentified cases are referred to as "multifactorial", a term that is rather ambiguous. It either means that some of the basic principles of teratogenesis still escape our attention, or the interpretation of some of the well known principles might be misleading. A third possibility is rather pessimistic. The development of the individual is so sophisticated and dependent on a delicate network of a multitude of factors mutually affecting each other that it is extremely prone to give rise to a plethora of spontaneous errors which are unpredictable and impossible to prevent. Nevertheless, the long and complicated history of scientific endeavour has yielded considerable present-day knowledge on causes and mechanisms of CDDs, a history whose beginnings date back to antiquity.

HISTORICAL CONTEXT

In ancient times, malformed individuals and unbelievable monsters have attracted human attention and they often served as prototypes of divine creatures endowed with magical capabilities. A congenital developmental disorder (CDD) is a phenomenon that has been accompanying humankind since its very beginnings. The earliest written records pertaining to CDDs originate from priests in ancient Babylonia. On clay tablets aged about 4,000 years, there are records written in cuneiform script describing malformations of the ears, nose, mouth, genitals, extremities, as well as various types of conjoined double monsters.

The relationship of the society towards such affected individuals varied over different historical epochs. Some cultures in antiquity considered cacomorphic (i.e., altered by disease) embryos as holy beings. In ancient Sparta newborn infants who were deemed by their family as unfit or bodily crippled, were thrown to predators into a chasm in the Taygetus mountain range. Romans would place infants in baskets on the Tiber, while in ancient India Hindus did the same on the Ganges. Carthaginians and certain Scandinavian nations killed crippled infants by drowning them in the sea. On the contrary, the attitude of rulers during the Renaissance period was rather benevolent towards their court jesters as they often served to amuse the ruling classes. Well known are the paintings depicting dwarfs by the Spanish painter Diego Rodriguez de Silva y Velasquez (1660) "Court Dwarf" and the "Boy from Vallecas". During the Middle Ages, people looked upon affected individuals relatively favourably. The ideology of Christian charity with its ideas that post-mortal blessedness could be achieved by many good deeds caused that many crippled individuals got food and certain charity care in cloisters. People always showed interest in crippled and deformed persons. For instance, the Russian tsar Peter the Great opened to the public his Kunstkammer in St Petersburg with his big collection of congenital cripples, conserved anomalies, deformed animals, surplus body parts, and other monstrosities.

Unique from the medical point of view was the case of two inseparable brothers, Lazarus and Joannes Baptista Colloredo, born in 1617 in Genoa. They were conjoined twins medically termed as *omphalopagus parasiticus*. From the chest of Lazarus, his imperfect brother, who never came to consciousness, grew out passively, like a doll. Colloredo was often showing up on markets and he became rich. The twins were the subject of a number of ballads, books and movies. In the 18th century, Marc Cazotte, the Parisian juggler and court jester, who was crippled by phocomelia (his underarms were missing), was endowed by extraordinary eloquence and the ability to chat in several languages. He died at the age of 44 and his skeleton is on show at the Duputryen Museum in Paris. Well-known is also the case of two Hungarian sisters, Helena and Judith, who were conjoined by their sacral region. They were born to a serf in the Komárno country in 1701. It has been an unwritten tradition for a long time that this developmental disorder occurred because during pregnancy their mother was watching a couple of dogs mating in an unnatural way. The term "Siamese twins" dates back to 1811, when in Siam (present-day Thailand), the twin brothers Chang and Eng were born. They were conjoined by their chest from the sternal bone down to the navel. They performed on markets and in circuses, mainly in the U.S.A. In 1840 they received American citizenship and adopted the surname Bunker. By pure chance they married two sisters and begot 12 and 10 children, respectively. They were performing as a feature in the Barum & Bally Circus until the end of their life. The twins died at the age of 63 years, 3 hours apart. The well-known Siamese twins Ružena and Jozefína Blažková were born in 1878 in Skrýchov near Bechyně in Bohemia. They were conjoined by their pelvic region, sharing common urinary organs, anus and vagina. As early as at the age of 11 years, they were famous world-wide by performing in amusement theatres in Europe and the U.S.A. (in the Karlín Theatre, Prague). The worldwide fame of the twins culminated in 1910 when one of the sisters, Ružena, gave birth to a son, František. Both sisters died at the same time, in 1922 at the age of 44 years.

Animal and human monstrosities sometime became an archetype of national symbols and coat-of-arms (heraldry symbols). For instance, the two-headed eagle was an emblem of the House of Habsburg, and at present it is the state symbol of Austria. In ancient civilizations the birth of a child heavily affected with a congenital disorder was often considered as "fatal" for the future of that given nation and empire. For instance, the birth of a two-headed infant in Constantinople under the rule of Theodosius was considered an instruction from the heavens to divide the Roman Empire into the Eastern and the Western Roman Empire.

HISTORICAL DEVELOPMENT AND MILESTONES IN TERATOLOGY

Besides antique myths and legends on monsters such as Minotaur (a creature with a bull-like head and a human body) or Chimeras (creatures with the head of a lion, body of a goat, and the tail of a snake), as early as in antiquity, there appeared the first attempts to provide exact descriptions of congenital malformations that can be designated by modern terminology. Ancient Chaldeans, for instance, described up to 62 types of various monstrosities, while ancient Egyptians, around 3,000 B.C., described a disorder in bone growth causing dwarfism, the so-called achondroplasia. The Greek physician and founder of medicine as a scientific discipline, Hippocrates of Kos (468–368 B.C.), was the first to describe the accumulation of body fluid in the brain, so-called hydrocephalus. The occurrence of these bodily abnormalities was for thousands of years

attributed to astral, divine, or other supernatural forces. More rational explanation of the causes of such monstrosities started to appear later in history. For instance, the ancient Greek philosopher, mathematician and astronomer, Thales of Miletus explained the occurrence of centaurs by hybridization, mating between a man and a mare, while the Greek philosopher Empedocles of Acragas (about 495–435 B.C.) explained the cause of monstrosities by lack, excess, or low motility of sperms. The philosopher Democritus of Abdera (about 460–370 B.C.) saw the cause in the admixture of sperms of two men in the genitals of a single woman. Another explanation was the assumption that such affected children were the consequence of sexual relationship between a woman and the Satan. It was the revenge of Satan for such a divine creation like the human body. Empedocles and Democritus attributed no formative functions to the pregnant woman in the generative process. In their opinion, the woman is merely a "specialized incubator". On the contrary, Hippocrates of Kos and Aristotle of Stageira (384–322 B.C.) stated that the mother contributed to the formation of her foetus, and their theories regarding teratogenesis included, besides the appearance of seminal disorders, also errors in the female element. In connection with CDDs, the most widely spread idea was that visual perceptions of a pregnant woman might directly form the appearance of her child. In ancient Greece it was believed that a woman would give birth to a more beautiful child if she were looking at beautiful statues as much as possible. Ambroise Paré (1510–1590), the "father of French surgery", explained e.g. the birth of frog-faced infants as a consequence of treating fever in pregnant women with holding a cold frog in their hands.

Further development of knowledge and society led to the recognition that causes of CDDs might be various mechanical effects, foetal diseases, and retarded or arrested development of the embryo and foetus.

Fig. 1. A cyclopic pig with proboscis. Engraved by N.F. Regnault in Moreau de la Sarthe, Paris, 1808.

However, it took a long time until these hypotheses could also be confirmed experimentally. The work of W. Harvey (1578–1657), "Exercitationes de generatione animalium" (1651), represents a significant milestone in the history of experimental morphology. He believed that man's semen, upon entering the uterus, turns into an egg matter from which the embryo is formed. He used the term "developmental arrest". Harvey was also considering the effect of external factors on prenatal development. For example, he stated that a constrained uterus or an incorrect posture of the pregnant woman resulted in various foetal malformations, or that a strong pressure of the uterine walls on the foetus caused so-called clubfoot. The Berliner anatomist and biologist Caspar Friedrich Wolff (1733–1794) is considered the founder of modern embryology (Wolf, 1759). The results of his experiments on chickens were described in his monograph "Theoria generationis". In his study on the intestine, the term "germ layer" was coined that has been in use to this day. Albrecht von Haller (1708–1777), a Renaissance-type man, presented a general and systematic overview of the scientific literature on malformations in his book "Operum anatomici argumenti minorum – tomus tertius" in which he rejected many older unacceptable and "naive" explanations of the development of CDDs. In 1758 he was the first to describe the development of the chicken heart. Etienne Geoffroy de Saint-Hilaire (1772–1844), the "first experimental teratologist", studied animal monstrosities in great detail. His work was continued by his son, Isidore Geoffroy de Saint-Hilaire (1805–1861), who was the first to introduce the term "teratology" to scientific terminology. The epoch of systematic teratology began with the edition of the 3-volume book by I.G. Saint-Hilaire „Histoire générale et particulière des anomalies de l'organisation chez l'homme et les animaux, ouvrage comprenant des recherches sur les caractères, la classification l'influence physiologique et pathologique, les rapports généraux, les lois et les causes des monstruosités, des variété et vices de conformation, ou traitė de tératologie I–III." Paris: Bailière in 1832–1837. Based on comparative anatomy, I.G.Saint-Hilaire classified human and animal malformations. Saint-Hilaire, both father and son, are considered founders of experimental teratology. In 1855, Camille Dareste published a monograph entitled "Mémoire sur l'influence qu´exerce sur le dévelopment du poulet", in which he discussed the modes of artificial induction of monstrosities (first of all by mechanical impulses during incubation of the hen's eggs). With his studies Dareste became one of the pioneers in experimental teratology. Rudolf Virchow (1821–1902), the famous German physician and scientist, gathered a unique collection, the "Museum of Pathology", counting up to several thousands of rare developmental disorders of the human body in the Berliner hospital Charité. He coined the medical

term "pathological physiology". The anatomist Ernst Schwalbe in his book "Allgemeine Missbildungslehre (Teratologie) Eine Einführung in das Studium der abnormen Entwicklung. Die Morphologie der Missbildungen des Menschen und der Tiere", Jena: Fischer (1906) defined the expression "teratogenic termination point" (i.e. the latest period in foetal development still enabling the occurrence of malformations. In his opinion, a malformation was a change in morphology of one or several organs, organ systems, or the whole body of the foetus exceeding the variation range of any given species. Charles R. Stockard in his book „Developmental rate and structural expression; an experimental study of twins, "double monsters" and single deformities, and the interaction among embryonic organs during their origin and development" (1921) introduced the term critical period by which he referred to the extraordinarily sensitive period of development of an organ or organ system. Morphogenetically, this period is characterized by rapid growth and development of the respective organ. During this period, CDDs can be induced without damaging the foetus as a whole because the other parts of the foetus are not in a period of intense cell proliferation.

During subsequent years, a huge number of experiments were carried out in experimental teratology connected with many names and a vast body of knowledge. Josef Warkany is considered father of experimental teratology. In the 30s and 40s of the past century, he was the first to prove that CDDs can be induced by exogenous factors also in mammals. Teratology has become an interdisciplinary science exceeding by far the originally strict classification as part of pathological anatomy. In this period, teratology already had a developmental background, case history, correlation and experiment. Modern experimental teratology is apparently based on knowledge acquired in experiments with eggs of fishes, amphibians and birds. An advantage of experiments with non-mammalian foetuses is that they are easy to manipulate, the influence of the maternal organism can be ruled out, and moreover, they provide a deeper knowledge regarding the course of normal developmental processes compared to mammalian embryos (Warkany, 1965). As to extrapolating the knowledge obtained in animals to man, a significant fact was the employment of mammals in experimental practice as model test systems. It is assumed that species that are closer related will have more comparable vital processes and responses to external factors. Hale (1933; 1935) induced anophthalmy and cleft palate in pigs suffering from vitamin A hypovitaminosis over a long period. Later Warkany & Nelson (1940), Warkany & Schraffenberger (1944), and Wilson *et al.* (1953) also found that hypovitaminosis, as well as other external factors, may cause disorders during intrauterine development of the foetus. In further experiments, various physical factors have been used (radiation, changes in temperature, hypoxia), hormones (estrogens, androgens, cortisone), hypovitaminoses (riboflavin, folic acid), hypervitaminoses (vitamin A, D), but also drugs and a number of other chemical substances. In this period, publications appeared reporting on the effect of the environment and genetics, as well as their mutual combinations, as the causes of malformations in experimental animals.

Despite the extensive work done in the field of experimental teratology, three significant clinical discoveries became milestones in the study of CDDs in man:

1. Post-conceptive radiation therapy of the pelvic organs results in microcephalus and other developmental disorders in the brain (Goldstein & Murphy, 1929).
2. Rose-rash overcome in the first trimester of gestation induces congenital malformations (Gregg, 1941).
3. Thalidomide intake between weeks 3–8 of gestation causes amelia and phocomelia (Lenz, 1961).

The major events that contributed to our knowledge in teratology prior to the thalidomide catastrophes are listed in Table 1.

Tab. 1. Historical events in modern teratology (pre-thalidomide period) Adapted according to Schardein (1988).

1905	The first experimentally induced developmental toxicity in mammals. Embryonic lethality induced by X-rays in cats (Tousey).
1921	The first experimentally induced teratogenicity in mammals. Disorders in limbs in pigs induced by lipid diet (Zilva *et al.*).
1929	The first description of malformations in humans caused by exogenous factors. Microcephalia caused by X-ray irradiation of the pelvis (Goldstein & Murphy).
1935	Recognition of food deficiency leading to malformations in animals. Eye disorders in pigs due to hypovitaminosis A (Hale).
1937	Hormones cause alterations in sexual differentiation in animals. Masculinisation of female foetuses in mice due to the action of androgens (Raynaud).
1941	Report on virus-induced human malformations. Rose-rash induced eye disorders (Gregg).
1944	The first evidence of post-natal effect following prenatal administration of a chemical substance. Decreased learning ability in rats caused by the application of sodium bromide (Hamilton & Harned).
1948	General recognition of chemically induced teratogenicity. Experiments with alkylating agents (Haskin) and trypan blue (Gillman *et al.*).
1952	The first report on malformations caused by drugs in humans. Multiple malformations in foetuses caused by aminopterin (Thiersch).
1959	The first report on human malformations induced by environmental pollutants. Disorders of the central nervous system and in dentition caused by methyl mercury (Kitamura *et al.*).
1961	Thalidomide-induced embryopathy.

The aim of experimental teratology in the post-thalidomide period has been the exact explanation of causes and mechanisms of the rise of CDDs. Further fundamental terms in teratology have been defined along with main principles of teratogenesis. Brent (1969) divided the tasks of experimental teratology into four main areas:

1. Research into normal morphogenetic processes and their disturbance by a teratogenic agent.
2. Elaboration of experimental models for the study of interaction between genetic and non-genetic components of embryonic development.
3. Identification and investigation of further (novel) causes of embryopathies.
4. Development of novel methods for testing pharmaceuticals and other xenobiotics, evaluation of conditions of the external environment of the human population with respect to their potential teratogenicity.

Wilson (1973) defined teratology as a science dealing with causes, mechanisms of occurrence and forms of deviations of either structural or functional nature. This concerned the study of adverse effects of the external environment on developing systems, such as germ cells, foetuses, and non-adult individuals during postnatal development. Wilson formulated a concept of six main principles of teratology that are generally accepted to this day:

I. Sensitivity to teratogenesis depends on genotype of the embryo, and on the mode by which its interaction with factors of the external environment takes place.
II. Sensitivity to teratogenesis depends on the period of embryonic development in which the embryo is exposed to the action of adverse factors (critical period).
III. The teratogenic agent acts by a certain specific mode (mechanism) on developing cells and tissues of the embryo and induces a sequence of abnormal developmental processes.
IV. The access of noxious exogenous factors to the developing tissues depends on the nature of these factors (agents).
V. An abnormal development may finally manifest as death, malformation, growth arrest, or a functional disorder.
VI. The occurrence and severity of abnormal development is enhanced with increasing doses of the teratogenic factor ranging from none to lethal effect.

Over the past more than 30 years, a number of sophisticated preventive measures have been created employing DNA diagnostics or high throughput ultrasound apparatuses, and yet the occurrence of CDDs remained unchanged worldwide.

In Slovakia e.g., the data on the occurrence of CDDs have been recorded since 1967 in conjunction with the state-granted system of registering live-born neonates. With 60 types of CDDs, the system was reintroduced in 1975 using the so-called "Kučera's system of reporting congenital disorders" (Böhmer & Šticová, 1997). In 1994 the system of reporting was modified in agreement with the international system of registering CDDs in live-born neonates and infants within their first year of life, categorized according to the International Classification of Diseases (Böhmer, 2004). Böhmer *et al.* (2009) analysed CDDs in Slovakia between 1997 and 2005. In this period, the mean occurrence of reported CDDs was 2.54 % of all live-born neonates. The spectrum of CDDs was as follows: CDDs of the CNS and spina bifida, CDDs of heart and blood vessels, cleft lip and palate, CDDs of bones, joints and muscles, and syndromes due to chromosomal aberrations. The occurrence of CDDs in Slovakia is in agreement with the European system of registration EUROCAT (Böhmer *et al.* 2009).

The significant progress in medicine with the advent of modern diagnostic procedures on the one hand, and the "constant" occurrence of CDDs in the human population on the other, stimulated Warkany (1981) to formulate his famous and frequently cited statement: "...something may be wrong about the way we are approaching the problem of congenital malformations..." Despite thousands of articles in renowned journals, hundreds of seminars and conferences, and the constant effort of teratological information services, his statement has remained valid until the present days.

Prof. Richard Jelínek, a Czech teratologist, came with the "revised" principles of teratogenesis (Jelínek, 1988). His concept was based upon the idea that the basic unit of individual development and teratogenesis is not the single cell but the morphogentic system defined as a set of cell populations carrying, creating and performing the program for the development of definitive body parts. The development of any structure begins with proliferation necessary to produce a critical amount of cells that in the beginning have a similar pattern of gene expression. Gene expression is modified by signals coming from the cell surrounding, mainly from neighbouring cells that take different positions during their growth. This results in a different signalling followed by modification of the existing gene expression manifested as differentiation, which creates a more exact program performed again by proliferation, active migration, cell association, or cell death (the four fundamental processes of morphogenesis). Thus differentiation is a secondary phenomenon depending on changes in cell position along time and spatial coordinates of the developing system. By this process the originally omnipotent stem cells give rise to a line of multipotent cells, progenitors, precursors, or mature cells. However, some of the multipotent cells remain unchanged, and become a source for replacing and/or regenerating damaged cells.

Of the modified principles, the following should be mentioned here:

1. Independently of the limiting stages of ontogenesis, called the critical periods of morphogenesis (Stockard, 1921), the only periods during which exogenous agents are capable of inducing specific developmental defects, there are also so-called sensitive periods, defined as the phases of development during which differentiated cells become sensitive to the administration of a given toxic substance. The extent of any sensitive period depends on the dose and the physicochemical properties of the agent. The agent is only capable of inducing developmental defects if the critical and the sensitive period overlap.
2. In case of chemical agents, the conditions of exposure of the morphogenetic system are given besides their dosage also by their pharmacokinetics and biotransformation in the maternal body. These characteristics exhibit considerable intra-species, inter-species, as well as individual differences, depending, among others, also on the genetic background of the mother.
3. While the manifestations of a deviant development (e.g. malformation, growth arrest, death) in their sum always show a positive dose-dependent response, this is by far not the case for congenital defects. At the beginning, the occurrence and severity of these defects grows with the intensity of the embryotoxic impulses, however, after having reached a certain level, their absolute number begins to decline. Malformed embryos are gradually replaced with dead ones. It is therefore better to prefer the universal term embryotoxicity to designate all manifestations of a deviant development.

STATE OF THE ART

At present it is known that all responses of the cell are mediated through its genome. Due to this, a majority of congenital defects have a genetic basis ranging from the role of the respective dominant and/or recessive genes up to the interactions between multiple genes and environmental triggers (Rychter & Jelínek, 1978; Jelínek, 2005). Mendelian heredity is common in cases of congenital metabolic disorders that are based on a mutation in the sequence encoding a certain enzyme. The second group is much more complicated. As the adverse factors can affect an unlimited number of genes, it is necessary to define the key ones which regulate the development of the embryo and those that are responsible for the pharmacokinetics and biotransformation in the maternal organism. Molecular mechanisms have also been implicated in some of the known teratogens, such as thalidomide, retinoids and valproic acid (Finnel *et al.* 2002). It is also presumed that genetic differences in the metabolism of folic acid caused by methylene reductase polymorphism are responsible for the increasing risk for neural tube defects in women treated with anti-epileptic drugs. Such results are very important as they contribute to a better understanding of the general (though at the same time rather ambiguous) term "multifactorially induced congenital disorders". It is assumed that a deeper insight into the changes of gene expression induced by teratogens enables better diagnostics and prevention of congenital defects originating in the interactions between a given noxious factor and specific allelic variants. Genetic variants have been identified in more than 20 human enzymes of metabolizing drugs, which are the basis for different responses of individuals to therapy, and also for different teratogenic risks of their offspring (Polifka *et al.* 2002).

Based on the principles of teratology, it is currently generally accepted that for the evaluation of a given substance as a risk factor for man we need:

a. Case reports describing repeatedly a typical pattern of malformation (syndrome).
b. At least two large and well controlled epidemiological trials.
c. Results of tests on laboratory mammals based on the principles of Good Laboratory Practice (GLP) and WHO criteria.

Informations on the adverse effects of substances are, as a rule, obtained in the order as follows: data from studies on animals, case reports, and epidemiological studies. In newly marketed drugs, the test results on animals, basic toxicologic data, and data on pharmacokinetics are available largely only from the manufacturers. New drugs are usually not recommended during gestation and/or around delivery due to the unknown risks both for the mother and the developing foetus. However, if pharmacotherapy of a pregnant woman is unavoidable, there is a chance to compare the ratio between embryotoxic doses assessed in experiment and the planned doses for humans, as well as the ratio between embryotoxic doses and the doses toxic to the mother. Despite the fact that this mode of evaluation is biased by species specificity, it does provide a basic orientation. In other cases, data are obtained from unwanted exposures of pregnant women to adverse factors. It is true that a majority of the known teratogens were discovered by competent physicians on the basis of case reports rather than by the application of good scientific methods (Jelínek, 2005).

Experts have pointed out the fact that the adverse effects of a number of novel chemical substances have not yet been investigated, including those that are commonly used (artificial fertilizers, solvents, new drugs). In the risk regions there is a dangerously increasing concentration of sulphur trioxide, nitrogen oxides, hydrogen sulphide, and other teratogenic factors (Graca & Machado, 1995; Bishop & Sloane 1997; Longo, 1980; Brent, 2004). These negative life conditions create a suitable milieu for the development of CCDs, increased tumor rate and the rate of prematurely delivered foetuses (Wigle *et al.* 2007). In the fight with this deleteri-

ous situation, prenatal diagnostics plays a crucial role. With the development of screening methods of prenatal diagnostics, the prenatal detection of some CDDs has been improving, resulting in their declining incidence in live-born infants. Presently they predominantly concern defects of the neural tube (anencephalia, spina bifida, encephalocoele), cleft disorders of the abdominal wall (omphalocoele, gastroschisis), and certain cardiac disorders (syndrome of left heart hypoplasia), congenital malformations of the kidney (agenesis and renal hypoplasia), and chromosome related defects, such as Down's syndrome (Šustrová, 2007; Chovancová, 2007; Garne *et al.* 2001; Forrester & Merz 2006; Johnson *et al.* 2009).

In the detection of the occurrence of CDDs, modern methods of postnatal diagnostics also play an important role (Cohen-Overbeek, 2010; Poretti *et al.* 2010; Hourrier *et al.* 2011; Shaffer and Bejjani, 2011). It is first of all the employment of ultrasound techniques and the recently introduced imaging methods – computer tomography (CT), nuclear magnetic resonance (NMR), and other imaging methods employing radionuclides. By using these modern approaches it is possible to improve early diagnosis in affected individuals, whose survival chance used to be relatively low, by detecting CCDs, mainly those of the CNS, heart, kidney and the digestive tract.

Considering the future of teratology and the main problems of prevention, evaluation and detection of potential environmental embryotoxicity and the possible effects on the human population, it is important to cooperate effectively in all related disciplines. Let us quote here the founder of Czech teratology, Prof. Richard Jelínek regarding the development and perspectives of this branch of science. In his words, in the field of basic teratological research it is necessary to:

1. Elucidate the fundamental laws of development of the organism and of its direction on the level of cell populations.
2. Understand the mechanisms of normal and pathological development.
3. Know the fundamental principles of directing sets of cell populations (morphogenetic systems) and the biochemical basis of information flow among individual cells that actively cooperate in the formation of fundamentals of organ systems, organs and organ components.
4. In the mechanism of teratogenesis it is necessary to determine the volume of cell reserves of morphogenetic systems that are decisive in the beginning of development and intentionally direct the prevention of the respective types of malformations to enhance these reserves.
5. Study new aspects of causes of the so-called civilization diseases. It is highly probable that the origin of some of them (e.g. hypertension, diabetes mellitus type II, metabolic syndromes, neuropsychic, autoimmune and oncological diseases) roots in prenatal ontogenesis, i.e. in the period in which the postnatal functions, limits of its adaptability and output are formed.
6. The embryotoxic and teratogenic factors of the environment should be investigated not only separately, but also under conditions of the action of complex factors that are likely to be the most frequent cause of occurrence of CDDs.
7. In the field of testing embryotoxic effects, the pharmacokinetic parameters of the respective experimental systems should be investigasted, and the present testing methods should be completed by suitable alternatives that might enable a deeper analysis of effects, as well as the screening of metabolites specific to man.

The results of clinical, epidemiological and experimental studies clearly show that adverse environmental conditions during critical developmental stages of gestation and postnatal development induce changes resulting in the occurrence of CDDs either of structural and/or functional nature (Schardein 2000; Kalter 2003; Dolk & Vrijheid 2003; Brent 2004; Ujházy *et al.* 2005; Genuis 2006; Hood 2006; Mattisson 2010). Owing to the improved methodological approaches of preclinical assessment of new drugs, the thalidomide tragedy has not occurred again. A number of serious environmental pollutants have been identified and the role of psychosocial and socio-economical factors in the development of CCDs is better understood. Experiments with animal models have revealed several hundreds of teratogens, nevertheless teratogenicity in man could be confirmed only in 20 substances (Shepard 1973; Kalter 2003). Some of the problems of teratogenic effects mediated by the exposure of the workplace environment of the father have been elucidated (Stefankiewitz *et al.* 2006; Jurewicz *et al.* 2009; Mattison 2010). Nevertheless, it is necessary to further improve research approaches whose results would contribute to the elaboration of effective preventive, diagnostic and therapeutic strategies that would positively affect a healthy development of future generations.

FUNCTIONAL AND NEUROBEHAVIORAL TERATOLOGY

In light of further development in teratology, functional disorders of various degrees of severity should also be mentioned. During their development, the nervous, endocrine, immune and reproductive systems represent a sensitive target for the action of various chemical substances and other factors. According to Prof. Günter Dörner, these systems form a mutually functionally interconnected neuroendocrine-immune system. During critical developmental stages, various hormones, neurotransmitters and cytokines play a key role in the functional development of the respective physiological systems as so-called ontogens, or develop-

mental signals and organizers. Various environmental factors may, directly or indirectly, affect the respective developmental processes, such as cell proliferation and migration, development of neurites, myelinization, synaptogenesis, or apoptosis. However, they also can act together with the activity of biologically active signal substances and subsequently they can cause their non-physiological concentrations. These changed concentrations of ontogens may act as so-called endogenous or functional teratogens (Dörner 2004).

The developing brain is extremely sensitive to the action of chemical substances and other influences. Functional alterations in the brain do not manifest immediately or shortly after birth but usually later, during childhood, adolescence or even during adulthood. Recent studies have shown some changes, mainly in the field of behaviour and emotions, which would become manifest only in the later process of getting old (Winneke 2011; Masuo & Ishido 2011). Some of the functional disorders/changes need not manifest under basic conditions, but they appear after the action of certain physiological burdens, such as alcohol and drug abuse, polluted environment, or the action of excessive stress. The functional changes may appear as behavioural, emotional, cognitive, and mental disorders (Dubovický 2010). The first experimental proof that a chemical substance can adversely affect the development of cognitive functions was reported by Hamilton & Harned (1944). They found that sodium bromide administered prenatally caused decreased ability of spatial learning in adult rats. Werboff & Gottlieb (1963) were the first to present the idea that chemical substances acting during prenatal development can affect the behaviour of an individual during the postnatal period, and they formulated the fundamentals of a new teratological discipline, the so-called behavioural teratology. During the 70s and 80s of the 20th century, several chemical substances were identified along with other factors acting as functional and/or behavioural teratogens, e.g. alcohol, certain addictive substances, heavy metals, X-ray radiation, and environmental pollutants (Grandjean & Landrigan 2006). The term behavioral teratology has not been generally accepted. Some professionals in the field considered teratology largely the domain for severe structural malformations. At present, the terms neurobehavioral teratology or developmental neurotoxicology have been preferred. These terms, however, represent a more complex study of causes and mechanisms (the so-called toxicity pathways) of damage to the developing brain (Bushnell *et al.* 2010). Developmental neurotoxicology includes research on several levels, such as brain histopathology, while assessment of behavior (behavioural teratology) is only a part of the relevant components.

Further development of neurobehavioral teratology is considered a very important issue. It is associated with the so far unexplained increasing rate of psychic and behavioural disorders on the one hand, and with excessive chemization and the action of excessive stressful stimuli on the other. According to several experts, the extremely increasing number of individuals with an autistic spectrum of disorders recorded in the U.S.A. over the past 20 years cannot be explained only by improved diagnostics and changed diagnostic criteria (Kim *et al.* 2011). It is assumed that a number of chemical substances and their mutual interactions, with no negative effect on the adult brain, may over their long-term action negatively affect the developing brain even at very low concentrations. Functional changes in the brain may become subsequently manifested as various behavioral, emotional, or cognitive disorders.

SUMMARY

Although at present a number of modern technical approaches are available, such as DNA diagnostics or the modern imaging methods, the rate of CDDs has remained actually the same in the human population. It thus appears mandatory to work intensively on further improvement of preventive, diagnostic, and therapeutic approaches and techniques that could positively affect the health of the population in the future. An interdisciplinary approach is a prerequisite, along with cooperation of experts from clinical and experimental practice, the study of causes and mechanisms of development of abnormal structural and functional development on all levels, both *in vitro, in vivo*, as well as *in silico*. For the experts working in the field of abnormal development and its consequences, there are still a number of possibilities to investigate the wide spectrum of so far unanswered questions and problems. For instance, it is the study of interactions between the genome and the environmental factors, the investigation of changes in gene expressions and their phenotypic consequences (epigenetics), or a joint action of several potential teratogens that await further insights into these issues.. In association with the marked increase in the number of mental and behavioral disorders in industrialized countries of the world it is necessary to investigate whether this phenomenon is caused by excessive chemization of our society or by progress in modern diagnostics and therapy, or both. The inability to significantly decrease the long-term constant occurrence of CDDs in the population and the increase in certain forms of functional disorders is still a great challenge to all those experts who devoted their professional lives to the research into various aspects of abnormal development in man.

ACKNOWLEDGEMENT

The study was supported by the VEGA grant 2/0081/11.

REFERENCES

1 Bishop JB, Witt KL, Sloane RA (1997). Genetic toxicities of human teratogens. Mutat. Res. **12:** 9–43.
2 Böhmer D (2004). Analysis of appearance of congenital malformations in live-born in Slovak Republic. Gynekol. prax **2:** 34–36 (in Slovak).
3 Böhmer D, Luha J, Martináková J, Repiská V (2009). Congenital developmental defects Slovak Republic (1997–2005). Biomedical Studies III. Slovak Biological Society SASc (Editors: L. Badalík, I.M. Tomo). Bratislava, (in Slovak) 44–46.
4 Böhmer D, Šticová A (1997). Territorial differentiation of congenital defects occurrence in Eastern Slovakia. 6th Demographic conference of the Slovak Statistical and Demographic Society: Birth-rate and selected aspects of the reproduction of the population. Textbook of the *in extensor* lectures (in Slovak) 118–123.
5 Brent RL (1969). Implication of experimental teratology. Cong. Malform. Proc. of Third Internat. Conf. Hague: 187–195.
6 Brent RL (2004). Environmental causes of human congenital malformations: the pediatrician role in dealing with these complex clinical problems caused by a multiplicity of environmental and genetic factors. Pediatrics **113**(Suppl 4): 957–968.
7 Bushnell PJ, Kavlock RJ, Crofton KM, Weiss B, Rice DC (2010). Behavioral toxicology in the 21st century: chalenges and opportunities for behavioral scientists. Neurotoxicol. Teratol. **32:** 313–328.
8 Chovancová D (2007). Neonatal screening. Pediatr prax **1:** 13–16 (in Slovak).
9 Cohen-Overbeek TE, Tong WH,Hatzman TR, Wilms JF, Govaerts LC, Galjaard RJ, Steegers EA, Hop WC, Wladimiroff JW, Tibboel D (2010). Omphalocele: comparison of outcome following prenatal or postnatal diagnosis. Ultrasound Obstet. Gynecol. **36:** 687–692.
10 Dareste C (1891). Production expérimentale des monstruosités. C. Reinwald et Cie Paris.
11 Dolk H, Vrijheid M (2003). The impact of environmental pollution on congenital anomalies. Br. Med. Bull. **68:** 25–45.
12 Dörner G (2004). Environment- and gene-dependent human ontogenesis, sociogenesis and phylogenesis (eco-geno-onto-socio-phylogenesis). Neuroendocrinology Letters **25:** 164–168.
13 Dubovicky M (2010). Neurobehavioral manifestations of developmental impairment of the brain. Interdiscip. Toxicol. **3:** 59–67.
14 Finnell RH, Waes JG, Eudy JD, Rosenquist TH (2002). Molecular basis of environmentally induced birth defects. Annu. Rev. Pharmacol. Toxicol. **42:** 181–208.
15 Forrester MB, Merz RD (2006). Use of prenatal diagnostic procedures in pregnancies affected with birth defects, Hawai, 1986–2002. Birth Defects Res. A. Clin. Mol. Teratol. **76:** 778–780.
16 Garne E, Loane M, de Vigan C, Scarano G, de Walle H, Gillerot Y, Stoll C, Addor MC, Stone D, Gener B, Feijoo M, Mosquera-Tenreiro C, Gatt M, Quesser-Luft A, Baena N (2004). Prenatal diagnostic procedures used in pregnancies with congenital malformations in 14 regions of Europe. Prenat. Diagn. **24:** 908–912.
17 Genuis SJ (2006). The chemical erosion of human health: adverse environmental exposure and *in-utero* pollution determinations of congenital disorders and chronic disease. J. Perinat. Med. **34:** 185–195.
18 Goldstein L, Murphy DP (1929). Ethiology of ill-health in children born after maternal irradition.II. Defective children born after postconception pelvic irradiation. Amer. J. Roentgenol. Radium Ther. Nucl. Med. **22:** 322–331.
19 Graca LM, Machado MH (1995). Teratogenic effects of exogenous agents. Acta Med. Port. **8:** 441–449.
20 Grandjean P, Landrigan PJ (2006). Developmental neurotoxicity of industrial chemicals. Lancet **368:** 2167–2178.
21 Gregg NM (1941). Congenital cataract following German measles in the mother. Trans Opthalmol. Soc. Aust. **3:** 35–46.
22 Hale F (1933). Pigs born with eyebolls. J. Hered. **24:** 105–106.
23 Hale F (1935). The relation of vitamin A to anophthalmos in pigs. Am. J. Opthalmol. **18:** 1087–1093.
24 Haller A (1970). Operum anatomici argumenti minorum, tomus tertius. Grasset, Lausanne, 1786. In: Congenital malformations: from Hippocrates to thalidomide by TVN Persaud. West Indian Med. J. **19:** 240–246.
25 Hamilton HC a Harned BK (1944). The effect of administratioin of sodium bromide to pregnant rats on the learning ability of the offspring. III. Three table test. J. Psychology **18:** 183–195.
26 Harwey W (1651). Excercitationes de Generatione Animalium, Typis Du-Gardianis; impensis Octaviani, Pulleyn in Coemetario, Paulino, Amsterdam and London.
27 Hood RD (2006). Developmental and reproductive toxicology. A practical approach. Second Edition. CRC Taylor& Fracis group 1–1149.
28 Hourrier S, Salomon L J, Bault JP, Dumez Y, Ville Y (2011). Prenatal diagnosis and management of foetal lung. Rev. Mal. Respir. **28:** 1017–1024.
29 Jelínek R (1988). The principles of teratogenesis revisited. Congenit. Anom. **28:** S145–155.
30 Jelínek R (2005). The contribution of new findings and ideas to the old principes of teratology. Reproductive Toxicology **20:** 295–300.
31 Johnson CY, Honein MA, Hobbs CA, Rasmussen SA (2009). National Birth Defects Prevention Study. Prenatal diagnosis of orofacial clefts, national birth defects prevention Study, 1998–2004. Prenat. Diagn. **29:** 833–839.
32 Jurewicz J, Hanke W, Radwan M, Bonde JF (2009). Environmental factors and semen quality. Int. J. Occup. Med. Environ. Health **22:** 305–329.
33 Kalter H (2003). Teratology in the twentieth century. Congenital malformations in humans and how their environmental causes were established. Elsevier Sciences B.V. 1–290.
34 Kim YS, Leventhal BL, Koh YJ, Fombonne E, Laska E, Lim EC, Cheon KA, Kim SJ, Kim YK, Lee H, Song DH, Grinker RR (2011). Prevalence of autism spectrum disorders in a total population sample. Am J. Psychiatry **168:** 904–912.
35 Lenz W (1961). Kindlische Missbildungen nach Medikament – Einnahme während der Gravidität. Deutsche Medizinische Wochenschrift **86:** 2255–2256.
36 Longo LD (1980). Environmental pollution and pregnancy: risks and uncertaines for the fetus and infant. Am. J. Obstet. Gynecol. **137**(2): 162–173.
37 Masuo Y, Ishido M (2011). Neurotoxicity of endocrine disruptors: possible involvement in brain development and neurodegeneration. J. Toxicol. Environ. Health B. Crit. Rev. **14:** 346–369.
38 Mattison DR (2010). Environmental exposures and development. Curr. Opin. Pediatr. **2:** 208–218.
39 Polifka JE, Friedman JM (2002). Medicinal genetics: 1. Clinical teratology in the age genomics. CMAJ **167:** 265–273.
40 Poretti A, Meodet A, Ceritoglu E, Boltshauser E, Huisman TA (2010). Postnatal *in vivo* MRI findings in anencephaly. Neuropediatrics **41:** 264–266.
41 Rychter Z, Jelínek R (1978). Foundations of experimental teratology. Praha: Avicenum, 1–159 (In Czech).
42 Saint-Hilaire IG (1832–1837). Histoire générale et particuliére des anomalies de l'organisation chez l'homme et les animaux, ouvrage comprenant des recherches sur les caractères, la classification l'influence physiologique et pathologique, les rapports généraux, les lois et les causes des monstruosités, des variété et vices de conformation, ou traitė de tératologie I–III. Paris: Bailière.
43 Shaffer LG, Bejjani BA (2010). Development of new postnatal diagnostic methods for chromosome disorders. Semin. Fetal Neonatal Med. **16:** 114–118.
44 Shepard TH (1973). Catalog of teratogenic agents. The Johns Hopkins University Press Baltimore and London, 1–210.
45 Schardein JL (1988). Teratological testing: status and issues after two decades of evaluation. Rev. Environ. Contam. Toxicol. **102:** 1–78.
46 Schardein LS (2000). Chemically induced birth defects. Third edition revised and expanded. New York, Basel: Marcel Dekker Inc. 1–1109.
47 Schwalbe E (1906). Allgemeine Missbildungslehre (Teratologie). Eine Einführung in das Studium der abnormen Entwiclung. Die Morphologie der Missbildungen des Menschen und der Tiere. Jena: Fischer.
48 Stefankiewitz J, Kurzawa R, Drozdzik M (2006). Environmental factors disturbing fertility of men. Ginekol. Pol. **77:** 163–169.

49 Stockard CR (1921). Developmental rate and structural expression; an experimental study of twins, „double monsters" and single deformities, and the interaction among embryonic organs during their origin and development. Am. J. Anat. **28:** 115–263.

50 Šustrová M (2007). Down syndrome at the present. Pediatr. prax **4:** 202–205 (in Slovak).

51 Ujházy E, Mach M, Dubovický M, Navarová J, Brucknerová I (2005). Developmental toxicology – an integral part of safety evaluation of new drugs. Biomedical Papers **149:** 81– 82.

52 Warkany J (1965). Development of experimental mammalian teratology. In: Wilson J.G. Warkany J: Teratology, Principles and Techniques. University of Chicago Press, Chicago-London, 1–11.

53 Warkany J (1981). Prevention of congenital malformations. Teratology **23:** 175–89.

54 Warkany J, Nelson RC (1940). Appearance of skeletal abnormalities in the offspring of rats reared on a deficient diet. Science **92:** 383–384.

55 Warkany J, Schraffenberger E (1944). Congenital malformations of the eyes induced in rats by maternal vitamin A deficiency. Proc. Soc. Exp. Biol. Med. **57:** 49–52.

56 Werboff J, Gottlieb JS (1963). Drugs in pregnancy: behavioral teratology. Obstet. Gynecol. Survey **18:** 420–423.

57 Wigle DTY, Arbucle TE, Walker M, Wade MG Liu S, Krewski D (2007). Environmental hazards: evidence for effects on child health. Toxicol. Environ. Health B. Crit. Rev. **10:** 1–2.

58 Wilson J, Roth C.B. Warkany J (1953). An analysis of the syndrome of malformation induced by maternal vitamin A deficiency. Effects of restoration of vitamin A at various times during gestation. Amer. J. Anat. **92:** 189–217.

59 Wilson JG (1973). Environment and birth defects. New York: Academic Press, 1–304.

60 Winneke G (2011). Developmental aspects of environmental neurotoxicology: lessons from lead and polychlorinated biphenyls. J. Neurol. Sci. **308:** 9–15.

61 Wolf KF (1970). Theoria generationis. Diss. Halle, Germany 1759. In: Congenital malformations: from Hippocrates to thalidomide by TVN Persaud. West Indian Med. J. **19:** 240–246.