BIOLOGICAL COUNCIL

THE CO-ORDINATING COMMITTEE FOR SYMPOSIA ON DRUG ACTION

A Symposium on

EMBRYOPATHIC ACTIVITY OF DRUGS

Editors

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PREFACE

THE thalidomide tragedy has stimulated a tremendous amount of research into the factors responsible for congenital abnormalities, and has particularly focused attention on the dangers of drug administration during pregnancy. It is a mere five years since the Ciba Symposium on Congenital Malformations was held in London. At that meeting, Warkany whilst discussing the teratogenic effects of some drugs, stated '. . . we should be very cautious in making general statements about these drugs . . . if we are not careful, we may hear very soon that aspirin "causes malformations".'

The subject of congenital malformations has become of such general interest, in particular as a problem of drug toxicity, that it was felt the time was ripe for a new discussion.

The Symposium was organized by a Committee consisting of F. Bergel, H. Jackson, C. Lutwak-Mann, J. W. Millen, R. L. Smith (Secretary), R. W. Smithells, A. Spinks, R. T. Williams (Chairman) and D. H. Woollam. The following societies participated in the Symposium: The Anatomical Society of Great Britain and Ireland, The Biochemical Society, The British Association for Cancer Research, The British Pharmacological Society, The Physiological Society, The Royal Society of Medicine, The Society for Developmental Biology and the Society for Endocrinology.

It was held in the Botany Theatre of University College, London, and was attended by more than 400 members of the participating societies. We are grateful to the authorities of University College and also to the staff for their help. Grateful acknowledgment is made to the Wellcome Trust for a grant which enabled us to invite contributors from Europe and the

United States, and also to the Ciba Foundation for hospitality afforded to overseas visitors.

In view of the interest in the subject we thought it desirable to prepare the publication as rapidly as possible and we are indebted to Mr. J. A. Rivers and to J. & A. Churchill Ltd. for their helpful co-operation and advice.

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CONTENTS

PAGE

Session 8

Chairman: Professor The Rt. Hon.
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EMBRYOLOGICAL PRINCIPLES OF TERATOGENESIS

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With the rediscovery of Mendel's work in the early part of this century man's ancient interest in teratology began to be translated into a scientific study. Naturally, there was at first a tendency to overstress the hereditary component in the aetiology of birth defects, but in 1921 Stockard published a classic study in which he showed that environmental agents were capable of producing various types of monster among treated fish embryos. Despite this it was felt that the mammalian uterus provided the organism with a constant and stable environment which could not be influenced by agents known to be teratogenic in lower forms; consequently little experimental teratology was attempted in mammals until quite recently. In 1922 Bagg produced eye defects in the offspring of rats subjected to ionizing radiations during pregnancy but at that time his studies were not considered relevant to human pathology. It was only with the work of Hale (1933) on deformities produced by vitamin A deficiency in the pig that experimental mammalian teratology became established, and after Gregg's (1941) observations on the embryopathic effect of maternal infection with rubella, interest in the subject became widespread. Since then many active teratogens both naturally occurring and artificially produced have been described and considerable information, at first of a purely descriptive but more recently of a more fundamental nature, has become available. At

the same time there have been important advances in the understanding of normal embryology and genetics, particularly at the molecular level. As a result it has become possible to formulate more basic questions regarding the nature of the embryogenic processes deranged by teratogenic agents and it is with such problems that this paper is largely concerned. Wilson (1959, 1961) has put forward five generalizations applicable to experimental mammalian teratology and in the discussion which follows, a slightly modified form of these is used as a framework upon which current ideas concerning the embryology of birth defects are elaborated.

1. *The activity of a teratogenic agent depends upon the developmental stage at which it is applied to the embryo*

Though apparently self evident this generalization must be interpreted with care. Stockard (1921) was the first to call attention to it in a formal manner, when he postulated that deformities produced in sea minnows by hypothermia or hypoxia were due to arrest of growth at specific times during development. However, he regarded teratogens as non-specific agents which caused an arrest of growth at critical stages of development, implying that the time of application exclusively determined the type of malformation produced. With increased experience of diverse teratogens it is now clear that this is not so and that many agents produce unique, if overlapping, patterns of congenital defect.

Mammalian eggs exhibit considerable powers of regulation during cleavage and only with the onset of gastrulation do the majority of the cells become broadly determined. At the same time mass morphogenetic movements accurately localize differentiating cells and enable a large number of secondary phenomena to end in the morphological and functional differentiation of the individual. It is unlikely, therefore, that malformations will be

induced by environmental agents acting on the mammalian ovum prior to gastrulation, for damage done at this stage could be made good by the remaining (totipotent) embryonic cells. Apparent exceptions have been reported by Russell & Russell (1954), by Woollam, Pratt & Fozzard (1957) and by Rugh & Grupp (1959), all of whom were able to produce small numbers of abnormal offspring by irradiation of rats and mice before gastrulation. Harvey & Chang (1962) reported similarly for hamster embryos, while Smith (1957) using hypothermia to produce congenital defects in hamsters described one abnormal litter from a mother treated before implantation. Such findings might result from local or general effects of the treatment persisting to affect the embryo at later developmental stages; alternatively it is possible that irradiation might have damaged the chromosomes, resulting in the expression of an altered genetic potency. Nevertheless it is important to remember that the concept of regulation is quantitative rather than qualitative; differentiation is a gradual process with no definite beginning and for this reason it would be wrong to make too much of the distinction between pre- and post-gastrulation stages. All that can be said with certainty is that there is considerably less likelihood of induced malformation resulting from agents administered before rather than during or after gastrulation.

Once implantation of the ovum has ensured that the nutritional requirements for rapid growth and differentiation can be satisfied, gastrulation takes place. At this stage the embryo is highly susceptible to teratogenic agents, probably because a number of integrated processes are occurring simultaneously and interference with any or all of them could result in the production of a deformed embryo. The major malformations will result from agents active soon after implantation for at this stage the body axis and principal organ anlagen are formed; thereafter less severe effects, often compatible with extrauterine life, are the rule. Each organ might be thought of as having a susceptible period occurring early

in the formation of its primordium, but it must be borne in mind that these periods will vary with the teratogenic agent employed because the latter will determine which facet of the developmental process is disturbed. For example Fraser (1964) cites a two-hour nicotinamide inhibition by 6-amino-nicotinamide at 13 days of development as being maximally effective in preventing palate closure in the mouse (Goldstein, Pinsky & Fraser, 1963), whereas the period of maximum palatal sensitivity to X-rays lies between 10 and 12 days together with an earlier period at 8 days (Russell & Russell, 1954).

During late embryonic and foetal stages susceptibility to teratogens is reduced once more for the simple reason that most of the important structures have already formed when these stages are reached. Nevertheless development is not complete and changes which are sensitive to external agents continue to take place until after birth. Hicks (1954) has described radiation malformations in rats and mice which can be induced well into the neonatal period and it is conceivable that a number of teratogens might induce malformations at late stages of development by producing pathological degeneration.

Another aspect of the temporal effect of teratogenic agents which must be taken into account is the relationship of the time of treatment to the state of development of the materno-foetal exchange mechanisms. Before implantation, the ovum enclosed in the zona pellucida, is relatively impermeable to substances in its immediate environment and, for this reason, most teratogenic agents are incapable of attacking it. In this connection it is interesting to note that only physical agents have been found able to produce abnormalities when treatment is applied at this time. After nidation, pre-placental and placental mechanisms are constantly changing and the critical period of a teratogenic agent may be due to its ability to interfere with placental transport at one particular developmental stage. The point may be illustrated by reference to the dye, trypan blue, which reaches a peak of tera-

togenic potency in the rat at 8·5 days, and ceases to have any activity whatsoever after 10·5 days of pregnancy (Wilson, Beaudoin & Free, 1959). Organogenesis is certainly not complete by the 11th day of gestation in the rat and many teratogenic agents such as vitamin A excess (Giroud & Martinet, 1955, 1956) produce abnormalities when administered after this stage (Kalter & Warkany, 1959); significantly these defects (cleft palate, limb defects etc.) are of a type not usually observed after trypan blue treatment. The mechanism by which trypan blue produces its effects is not known but a number of hypotheses have been advanced (see review by Beck & Lloyd, 1965). Among these is the suggestion that the dye acts by inhibiting the nutritional function of the absorptive visceral layer of the yolk sac epithelium, in which tissue it is specifically concentrated. This epithelium provides the only pathway for embryonic nutrition until, at about the tenth day, a functional chorio-allantoic placenta begins to be established, making an alternative source of nourishment available to the conceptus. From this stage onwards trypan blue ceases to be teratogenic. Alternatively it has been suggested that the dye might act directly upon the embryonic tissues and that its uptake by the yolk sac is a protective mechanism which becomes fully effective at about 11 days when the latter completely surrounds the embryo except for the narrow yolk stalk. If either theory of action is correct then the critical period for the teratogenic action of trypan blue is related to the form and function of the foetal membranes rather than to events referable to embryonic development *per se*.

2. *Because they usually modify specific developmental events, individual teratogens tend to produce characteristic malformation patterns*

It is not proposed here to deal with the mode of action of particular teratogens nor to attempt to classify these and the many varieties of defect which they produce. To compile a complete

list of teratogenic agents is nowadays a formidable undertaking and the mechanisms by means of which the majority produce their effects are in any case unknown. For this reason it would seem more appropriate to examine those embryological events which may be disorganized by external agents with consequent production of congenital defects. In classifying these it must be stressed that the division of embryogenesis into a number of discrete events is entirely artificial; a teratogen acting at a single point might interfere with a number of the developmental processes to be discussed.

(i) Although it is difficult to give many specific examples, the possibility exists that certain environmental agents act directly upon genetic material causing changes associated with congenital defect. This might happen during germ cell formation—as Hertwig's (1942) work on the house mouse has demonstrated—or as a somatic mutation during later developmental stages. Gene mutations would be duplicated at cell division and tissues or organs with abnormal function as well as morphology might result. It is possible that inborn errors of metabolism, in so far as they may be produced by environmental agents, are the result of this type of effect. The rise in incidence of mongolism with increasing maternal age provides an example of chromosomal abnormality influenced by (unknown) environmental agents and reflected as a congenital defect.

(ii) Inhibition of cell division and cell growth occurring at certain developmental stages can readily be understood to produce congenital abnormalities. The primary target of agents which affect these processes will obviously be the most actively growing tissues and, in the simplest form, their effect will be the non-development or underdevelopment of the appropriate part. Frequently widespread secondary effects will complicate the issue as for instance in the case of renal agenesis due to absence of the ureteric bud, or the systemic effects following non-development of an endocrine gland. A number of growth inhibitors and antimetabolites have

been shown to be potent teratogenic agents and are among the few substances whose mode of action is to some extent understood.

(iii) Malformations may arise from errors in normal differentiation, often, though not exclusively, following breakdown of an inductive mechanism (*vide infra*). Danforth's short-tailed mouse mutant Sd (Gluecksohn-Schoenheimer, 1945) for example produces a variety of effects among which abnormal differentiation of the notochord is an invariable feature. The latter forms normally but eventually disintegrates with secondary effects involving the vertebrae and nucleus pulposus; abnormalities of the urogenital system are also present in these mutants. Other examples of genes interfering with normal differentiation are available and it would seem reasonable to investigate the mode of action of experimental teratogens to see if similar effects are involved. Conceivably, cases of true hermaphroditism without a demonstrable genetic cause might be included under this heading.

(iv) The various ways in which disturbance of normal inductive processes may give rise to developmental defects have been ably reviewed by Zwilling (1961). Anomalies could result from the operation of a number of mechanisms each of which might be initiated in various ways.

(a) *Distortion of the inductive pattern* leading, for example, to microcephaly or anterior duplication in the case of derangement involving the primary head organizer.

(b) *Failure of reactants to make contact.* Zwilling cites the example of anophthalmic mice described by Chase & Chase (1941); in these mutants the rudimentary optic vesicle fails to make contact with the overlying epidermis and it is presumed that induction of the lens does not take place even though the inducing and responding capacities of the two tissues normally involved are unimpaired. Could it be that many teratogenic agents by producing oedema of the head (Grabowski, 1963) might produce anophthalmia or microphthalmia by a similar mechanism?