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Aetiological factors in childhood neoplasia

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In considering possible aetiological factors in the causation of the solid tumours of childhood it is necessary to keep in mind a number of epidemiological facts. Some of the tumours apparently have their origin in intrauterine life, a number are associated with specific syndromes of somatic abnormality, and others show distinct familial tendencies. Teratomas have distinctive characteristics. A unified hypothesis for tumour induction in childhood seems unlikely to fit all observations, and some apparent relationships, for example, that between teratogenesis and carcinogenesis, need to be carefully examined. Transplacental carcinogenesis is another important concept, recently shown to be relevant in man.

In this review I shall consider some of these problems and attempt to indicate which fields of study are likely to be profitable. In 1961 Campbell *et al* found that 65% of children with malignant disease were beyond medical help when the diagnosis was established. Since then great strides in treatment have been made, but identification of available predisposing factors is obviously important and, in one instance (irradiation), may already have proved valuable. Before assessing any factor, however, it is necessary to consider the problems of identifying potential carcinogens in man. One of the best descriptions of these difficulties has been given by Lynch (1969), who points out that the mode, time, and intensity of carcinogenic exposure must be considered in detail and related to host factors, such as age, sex, race environment, occupation culture, geographical site, etc. It is fortunate that many of these interrelated variables are more readily controlled in studies of children than in adult work.

Radiation

For some time confident assertions have been made about the role of radiation, particularly in the first trimester, in the genesis of childhood malignancy (see Stewart and Kneale, 1970). Recently, Oppenheim

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et al (1975), in an excellent review, have questioned some of these views. Studies involving preconception or prenatal doses in the normal range of diagnostic procedures (given as 1-5 rads) mainly involve individuals irradiated in the course of examination of the mother's abdomen or pelvis; these have associated (preconception x-rays) with an increased risk of leukaemia and Down's syndrome, and (prenatal x-rays) with leukaemia and other malignancies, alteration of sex ratio, and increased death rate from all causes.

Oppenheim and his colleagues point out that these studies are almost always concerned with individuals whose x-ray examination has been carried out on the basis of a distinct medical indication, and that these indications may themselves be associated with an increased incidence of neoplasia. For example, leukaemia incidence has been reported to be influenced by maternal age, birth weight, socio-economic class, birth rank, history of abortion, etc, and these factors also affect the incidence of prenatal irradiation (Michigan survey of medical radiation exposure during pregnancy, 1961; Diamond *et al*, 1973). When non-selective irradiation studies (ie, routine pelvimetry and atomic bomb irradiation) are considered, there is little evidence of an increased risk of neoplasia although for equal doses atomic radiation is more harmful biologically because of its neutron component. Jablon and Kato (1970) found only one cancer death under 10 years in 1292 children irradiated *in utero* by an atomic bomb, and, using conservative estimates of dosage, found that this was less than the minimum number predicted by Stewart and Kneale (1970).

The general finding, that there is a significant discrepancy between the observed and expected frequency of tumours in studies of 'non-indicated' irradiation, suggests that the risks of radiation may have been overemphasized. In addition, in the last 20 years x-ray practices have changed radically in a way which will considerably reduce dosage. Recently, studies of leukaemia incidence suggest that there may be a reduction in the number of cases of leukaemia up to the age of 14 years (Adelstein and White, 1976). It seems that the importance of radiation as a childhood carcinogen may have been overemphasized, and undoubtedly few tumours are induced in this way. However, even a very small risk of this type is worth preventing by changes in technique, and a valuable decrease in x-ray tumour induction has probably already taken place.

Transplacental carcinogenesis

This phenomenon was well established in animals before it was noticed in man, Larsen having reported

pulmonary tumour induction by urethane given during pregnancy as long ago as 1947. In 1970 Herbst and Scully reported on an unusually high frequency of vaginal adenocarcinoma in a group of young women. Their mothers had a history of previous miscarriage and of treatment with oestrogens during the pregnancy, resulting in the birth of the affected female. More than 170 such cases have now been reported to the clear-cell adenocarcinoma registry, and the following general points have been established.

Administration of diethylstilboestrol between the eighth and 18th week of pregnancy affects the development of Müllerian derivatives. Vaginal adenosis (the presence of glandular epithelium in the vagina), ectropion, transverse vaginal ridges, and abnormal vaginal epithelium may be found. Herbst *et al* (1975) have reported on the critical time/effect relationship involved (see table I) and the relative frequencies of these defects in exposed and non-exposed females (see table II). These abnormalities are associated with the subsequent development of clear-cell carcinoma in perhaps 4/1000 exposed females, according to Lanier *et al* (1973).

The median age at presentation of the tumour is around 17 years with a range of 8-28 years. A valuable study of vaginal adenosis in these patients by Antonioli and Burke (1975) has shown that the epithelial cell type may resemble any derived from Müllerian sources (tubal, cervical, endometrial) and that these may be arranged in complex or simple glands. There is often extensive squamous metaplasia and submucosal inflammation. In approximately a quarter of patients there is no surface involvement; this is an important point if vaginal cytology is considered as a screening technique.

This demonstration of transplacental carcino-

Week of pregnancy	No. of cases	Vaginal adenosis	Cervical erosion	Ridges
< 8	22	16	22	5
9-12	39	19	36	10
13-16	42	11	34	11
> 17	30	2	21	4

Table I Timing of administration of diethylstilboestrol and relationship to effect on genital tract (according to Herbst *et al* (1975))

Finding	110 exposed +	82 unexposed +
Transverse vaginal ridges	22	0
Abnormal vaginal mucosa ¹	56	1
Biopsy-proven adenosis	35	1

Table II Diethylstilboestrol: comparison of exposed and control cases

¹Failure to stain with iodine

genesis in man illustrates a number of points which have been amplified by animal studies. Several general conclusions can be drawn (see Berry and Keeling (1976) for bibliography):

(1) A wide variety of types of carcinogens are effective (dibenzanthracene, methylcholanthrene, aflatoxin, nitrosoureas, aliphatic nitrosamines, ethyl carbamate, O-aminoazotoluene, etc).

(2) The type of tumour produced in the progeny of the test pregnancy is the same as that produced by the carcinogen acting in adult life.

(3) Tumours do not appear until considerable time has elapsed and usually not until after sexual maturity.

(4) There are distinct differences in the effects of compounds when administered at different times in pregnancy. If they are given during the period of organogenesis, malformation follows; however, carcinogenesis results from exhibition after histiogenesis has occurred. The explanation of this finding is simple. Many carcinogens require a metabolic step to convert a precursor compound to the active carcinogen. For example, the action of dialkylnitrosamine is apparently mediated by an alkylating metabolite produced by enzymatic demethylation of the original compound. Because the bulk of these active metabolites are extremely short-lived they are effective only in the tissue in which they are produced. Thus the specific organ/tumour relationship, generally reflecting that seen in adult life, awaits the development of the enzymes normally seen in that tissue.

The third of these four points suggests that if transplacental carcinogenesis is a widespread but undetected phenomenon in man, it is likely to produce tumours in the second or third decades of life, in view of both the delay in onset found in animal experiments and in the single established example in man. Perhaps the late presenting sub-populations of tumour of childhood are distinct in this respect; for example, there is a considerable disparity in mean age at presentation of nasopharyngeal and genitourinary rhabdomyosarcoma.

One recently reported experiment of Goertler and Loehrke (1976) is of interest here. The design of the experiment is shown in the figure. Dimethylbenzanthracene (an 'initiator' in terms of the classic Berenblum experiment) was administered to mice *post partum*. TPA (12-0-teradecanoyl-phorbol-13-acetate), a promoting agent, was then painted onto the dorsal skin of the suckling mice daily. The offspring were followed for a year and were found to develop tumours on the dorsal skin and in the stomach, large bowel, liver, kidney, lung, and bone. This experiment suggests that a number of alternative mechanisms of transmaternal carcinogenesis

Figure From Goertler and Loehrke (1976): DMBA—dimethylbenzanthracene; TPA—12-0-teradecanoyl-phorbol-13 acetate.

exist and that other modes of action or interaction compounds should be sought.

It is convenient to emphasize important differences between teratogenesis and carcinogenesis at this point before considering the relationship of malformations to tumours and the importance of genetic factors in childhood oncogenesis. Table III shows a number of differences between transplacental carcinogens and teratogens. The two processes appear to be biologically distinct and the assumption made by a number of authors that they are necessarily related seems to be unjustified. This point is important when the relationship between neoplasia and malformation is considered (*vide infra*).

Teratogen (esis)	Carcinogen (esis)
Few are transplacental carcinogens	All are teratogens
May act via mother via placenta directly	Act directly
Nature of anomaly produced time-dependent	Tumour(s) produced not variable with time
Histodifferentiation seldom affected, stage reached not critical	No effect until histodifferentiation manifest
Complex disturbance of growth and morphogenesis over wide field	Probably few cells affected
Effect usually immediate or rapid	Time interval for manifestation usually considerable

Table III Comparison of transplacental carcinogens and carcinogenesis with teratogens and teratogenesis

Genetic influences in childhood neoplasia

In a number of distinctly defined syndromes a well-defined pattern of familial tumour formation is found. Polyposis coli, the pleuriglandular syndrome, and xeroderma pigmentosum are examples of this type; in the latter condition, the mechanism of tumour is perhaps better understood than any other human neoplasm. Ultraviolet irradiation in the 280–320 nm waveband damages DNA in the epidermis and upper dermis. Pyrimidine dimers and possibly other compounds produced by photochemical reactions cannot be 'excised' from the DNA molecule by specific enzymes and are presumed to interfere with DNA replication. How this leads to tumour formation is uncertain, but it seems likely that a particular enzyme deficiency is responsible for oncogenesis in these patients (see Gianelli, 1976). These lesions will not be discussed here although certain childhood tumours occur in this way. Tumours associated with chromosomal disease, immunological disorders, and other abnormalities collectively account for relatively few cases of childhood malignancy. Familial occurrence of solid tumours in children has been extensively reported (see Berry and Keeling (1976) for bibliography of histologically verified cases). In most instances, however, embryonic tumours of childhood are not associated with other abnormalities (the syndrome of nephroblastoma and hemihypertrophy is exceptional (Miller *et al*, 1964)) nor with well-defined patterns of inheritance, such as that found in retinoblastoma.

There are two main schools of thought concerning the possible genetic mechanisms involved in childhood malignancy, the 'mutation' type of hypothesis (Knudson and Strong, 1972; Knudson, 1974) and the Innes (1972) hypothesis of polygenic determination. These two schemes may be briefly described as follows:

POLYGENIC THEORY

Innes has pointed out that children of a similar genetic background have the same relative frequencies of embryonic tumours when in differing environments (continents) and that these frequencies remain constant with time. He has suggested that the tumours are determined by genes in Hardy-Weinberg equilibrium, and that environmental factors can have little effect in view of the timescales of induction involved. Further, genes in this type of equilibrium will be widespread in the population, and the selection pressure of malignant disease which, until comparatively recently, would have killed most patients before reproductive life began, will not be manifest.

His evidence supports the hypothesis but is

probably based on too few cases for some tumours, as he himself points out. Retinoblastoma is difficult to fit into this scheme, as it is certainly not in genetic equilibrium (for further discussion see Berry and Keeling (1976)).

MUTATION THEORY

Knudson's model suggests that a mutation, or mutations, produce the essential change necessary for tumour development. He describes the frequently observed duality of tumour behaviour, eg, one type of retinoblastoma apparently inherited through a dominant gene, and another type accounting for about 60% of tumours without hereditary pattern (similar findings have been presented for nephroblastoma). He suggests that the pattern might be explained by the occurrence of two mutations, one in germ cells followed by one in somatic cells in bilateral cases, and two in somatic cells in unilateral cases. Thus, in the bilateral cases, all cells formed after the initial event would be 'carriers' and only one further mutation is necessary in a given cell; in the unilateral group, both mutations would have to occur in the same somatic cell. Assuming a risk of 10^{-6} mutations per gene/cell division (Albertini and De Mars, 1973), the hypothesis is tenable in view of the number of cells in the anlage and the number of cell divisions involved. However, not everyone would agree that mutation is necessary for carcinogenesis, and observations such as those of Fialkow (1971) on the non-uniformity of isoenzymes in tumour cells from heterozygotes do not provide unequivocal evidence of a single cell origin for all tumours. Perhaps more important, in a paper in 1974, Li and Jaffe reported on the progeny of childhood survivors of malignant disease. They found no evidence to support the Knudson hypothesis, but their data are certainly insufficient to refute it.

TERATOMA

These tumours have aroused a great deal of interest in those concerned with the origin of childhood tumours. Sacrococcygeal masses are much the commonest type (58 of 91 in a personal series (Berry *et al*, 1969)) and usually originate during intrauterine life. In general, we have found that the tendency to malignant change is greater when a high proportion of embryonic tissue is present. This is interesting, in view of the suggestion that gene-activated growth and differentiation control phenomena, rather than mutation, are important in oncogenesis in this type of mass. The importance of controlling genes was first appreciated after work on the experimental teratoma of mice initiated by Stevens (see review by Damjanov and Solter (1974)). Apparently malignant spontaneous tumours from the 129/J strain may be

found to form differentiated tissues when transplanted to new sites.

In a report of an elegant series of experiments, Mintz and Illmensee (1975) have amplified and extended these findings. It is evident that malignant cells from such tumours, having been maintained *in vivo* for eight years, are still capable of differentiation into many tissues with normal functional characteristics (eg, immunoglobulin production, haemoglobin synthesis, liver protein production, etc). When the cells are injected into normal blastocysts from mice of other strains, the mosaic animals thus formed may also contain tissues of tumour origin which are not known to occur in the original teratocarcinoma. The demonstration of the capacity of these malignant cells to form normal tissues, including germ cells (shown by transmission of a coat colour gene of tumour origin), is of great importance in the debate about the origin of teratomata.

Two recent articles express opposing views. Erickson and Gondos (1976) suggest that teratomas represent pathogenically activated oöcytes while testicular tumours may represent postmeiotic fusion events, resembling a fertilization-like process, and seek to explain the differing biological behaviour of the two neoplasms in this way. Their view has been disputed by Riley and Sutton (1975, 1976), who have proposed a 'mutation origin' hypothesis.

Malformation and tumour

Associations of malformations and embryonic tumours undoubtedly exist, but in many studies, in which an increased incidence of congenital abnormalities associated with tumours has been recorded, the incidence of malformation is in fact no greater than would be expected by chance (Sy and Edmonson, 1968). There are real difficulties in determining the true frequency of congenital defects in this type of work since some are found because of thorough investigation of a child with a tumour (for example, the discovery of renal anomalies at intravenous pyelography). Conversely, some neoplasms are found when a malformation is investigated or treated. Tumours may also be discovered incidentally at necropsy of a child with malformation (notably the occult neuroblastoma). In a study of 288 embryonic tumours (Berry *et al*, 1976), we found no increase in malformation rate except in the group of sacrococcygeal teratomata. When cloacal abnormalities were excluded (these were attributed to the presence of a rapidly growing mass in the pelvis in intrauterine life), this association disappeared.

There does not seem to be any reason to associate the general processes of tumour formation and malformation in view of the biological differences

between them (*vide supra*). Those associations which are well documented cannot account for the bulk of neoplasms and may be likened to the association between tylosis and oesophageal carcinoma whose relevance for the general problem of carcinoma of the foregut is uncertain.

How may one summarize this complex picture? Evidence concerning the role of radiation in childhood neoplasia has been recently scrutinized, and the findings suggest that its importance may have been over-emphasized. Genetic factors are undoubtedly of fundamental importance. Well-defined genetically determined patterns of tumour occurrence account for a small but significant proportion of childhood tumours. In the bulk of cases, genetic influences are important but in a manner which is not clearly defined. It is important to separate any well-defined syndrome from the majority of cases of childhood malignancy. It will be evident that this type of work may reveal other constant features in the remaining cases that may help to identify other aetiological factors, possibly environmental ones acting via the mother or placenta. Definitive evidence concerning a number of 'genetic' hypotheses will become available when the success of modern therapy for embryonic tumours permits a more extensive study of the offspring of survivors.

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