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EFFECTS OF LOW-DOSE PRENATAL IRRADIATION ON THE CENTRAL NERVOUS SYSTEM

Report of a Workshop

November 5-7, 1990

Battelle Seattle Research Center
Seattle, Washington

Sponsored by
U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

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AUTHOR'S PREFACE

Scientists are in general agreement about the effects of prenatal irradiation, including those affecting the central nervous system (CNS). Differing concepts and research approaches have resulted in some uncertainties about some quantitative relationships, underlying interpretations, and conclusions. Examples of uncertainties include the existence of a threshold, the quantitative relationships between prenatal radiation doses and resulting physical and functional lesions, and processes by which lesions originate and develop. A workshop was convened in which scientists with varying backgrounds and viewpoints discussed these relationships and explored ways in which various disciplines could coordinate concepts and methodologies to suggest research directions for resolving uncertainties.

The Office of Health and Environmental Research (OHER) of the U.S. Department of Energy (DOE) encouraged and supported this Workshop, which was held at the Battelle Seattle Research Center on November 5-7, 1990. The subject was developed through a sequence of synoptic presentations and discussions of status and knowledge in the various fields. This approach allowed assembling a limited number of scientists who had been concerned with aspects of prenatal irradiation and the CNS and who represented the several research approaches. To facilitate broader interactions, participation by scientists from basic neuroscience and molecular biology provided other perspectives, concepts, and approaches. In addition, pragmatic viewpoints were provided by representatives of agencies that might be involved in using current and future knowledge in their mission and in implementing future research to meet the needs of society.

This Workshop Report summarizes, in an extended fashion, salient features of the presentations on the current status of our knowledge about the radiobiology and neuroscience of prenatal irradiation and the relationships between them. It also provides the essence of discussions among participants about research needs, potential interdisciplinary interactions, and recommendations for future research. The report integrates information recorded in my notes and those of colleagues, written material provided by some participants, and audio tape recordings of the presentations and discussion periods. No attempt

was made to include full details of presentations nor to reference the several investigators and documents that were mentioned. Instead, the report summarizes the presentations and selected portions of the discussions so that they will serve as a synopsis of the current state of knowledge and of remaining questions, uncertainties, and future directions. Only a few diagrams, which were redrawn and combined into simplified composites, are included, as are limited references to recent reviews and general documents to assist those who wish to obtain further detail.

Clarifications were necessary to reconcile differences in terminology and nomenclature used during many presentations and discussions, and an attempt is made to use uniform conventions in this report. When the day of sperm detection in rodents was defined as gestation day one, stage at subsequent times of gestation will be indicated by preceding the numerical value with the abbreviation gd (gd 9). When it was denoted as zero days of gestation, the equivalent time will be presented with the abbreviation after the stated day (8 dg); the latter convention is used when the approach was not known. Most speakers expressed time of human pregnancy in terms of weeks after fertilization; an attempt was made to use this system throughout. Expression of radiation exposures or doses varied: R, rad, cGy, and Gy. For uniformity, all have been restated in gray (Gy) except when precluded by historical considerations.

Several questions and concepts were raised following some presentations, and were reiterated during discussion periods. Many of these broad considerations have been integrated into the closing section of the report, which summarizes the final series of discussion sessions and resulting research recommendations and approaches. My hope is that the report accurately reflects the tone of presentations, discussions, conclusions, and recommendations. For easy reference, the report is preceded by a summary of the state of knowledge and an abbreviated presentation of recommendations. An attempt was made to avoid personal biases, and apologies are offered for the inevitable errors and transgressions that may have been introduced into the report or summary.

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SUMMARY

The workshop traced the historical perspectives and summarized current knowledge on effects of prenatal irradiation on the central nervous system (CNS). Topics included normal and altered morphologic development of the CNS, its functional capacity during postnatal life in experimental animals, as well as results of epidemiologic analyses of data from clinical evaluations of human populations. Background presentations were followed by others that examined current knowledge about changes that occur at the cellular and biochemical level. Finally, descriptions of current approaches used by investigators in the neurosciences and in molecular biology to investigate development of the CNS were presented. This format led to extended discussions that identified areas of agreement, remaining questions, and reasons for discrepancies; considered broad implications; and finally made recommendations for integrated research approaches that would address the effects, disparities, and mechanisms.

The initial presentations documented that higher doses of intrauterine irradiation lead to morphologic and functional deficits of the CNS. These effects were observed in clinical and postmortem evaluations of both individuals and human populations and in experimental investigations of laboratory animals. Clinical evaluations in postwar Japan were consistent with these findings, and investigators detected children with reduced head circumference and/or mental retardation among those born to women who received the highest radiation exposures from the atomic bomb detonations. Subsequent analyses determined that there were definite relationships between dose, stage of gestation at exposure, and several measures of CNS dysmorphology and dysfunction in these survivors. This is the only sizable human group that has been evaluated by techniques of population statistics, and conclusions from these analyses serve as the root of many ongoing discussions. In essence, the questions have become whether dose-response relationships for mental retardation or reduced intelligence after exposure in the period from 8 through 16 weeks of gestation are linear, and whether there is a threshold for these effects.

In addition to statistical uncertainties these studies are affected by other problems. For example, a number of adverse health and emotional

stresses were experienced by the exposed population, which serve to introduce uncertainties into interpretations because of their possible adverse effects on prenatal development or synergy with radiation. One such stress is malnutrition, which may have an effect on birth weight and associated problems of prematurity. The irradiated mothers of mentally retarded children underwent several additional traumas during their pregnancy, including exposure to the blast and heat. In addition to potentially synergistic effects associated with maternal irradiation, there may have been interactions with the effects of other insults, including infection.

Studies of experimental animals corroborate that prenatal irradiation produces CNS lesions, that effects tend to be reproducible and dose dependent, and that stage of gestation at exposure determines the specific characteristics and relative sensitivities of most types of lesions. The altered CNS endpoints in animals ranged from malformations and disrupted histoarchitecture to functional defects of several behaviors, reflexes, and sensory-motor capabilities. The dose levels at which unequivocal permanent damage could be demonstrated, with essentially every endpoint reported, have been in the 0.15- to 0.25-Gy range, both in vivo and in vitro.

Techniques used in earlier investigations of morphologic alterations and functional decrements of the CNS following prenatal radiation are crude by current standards, but most details and interpretations are still accepted. Techniques with greater sensitivity have become available to define and correlate lesions with changes in neurofunctional and cognitive measures. These more recently determined endpoints, such as alterations of the cell cycle, histogenic details of neuronal migration, and quantified measures of neuronal and fiber alignment, all yield a similar threshold or no-detectable-effect dose range. The site and identity of cells that die and the time at which they die are being studied to define mechanisms and draw conclusions regarding response relationships. The times and mechanisms of cytolethal processes, removal of dead cells in migrating populations via phagocytosis or apoptosis, and the possibility of sequential death of "different" neuronal "stem cell" populations remain unanswered questions.

When appropriate scaling for gestational stage is used as the basis for comparisons, there is remarkable similarity between the development of the

human brain and that of other mammalian species. Some categories of functional deficits, such as altered behavior and seizures, are similar and perhaps identical throughout. There is quantitative consistency between radiation effects in humans and animals; the threshold range across species seems to be about 0.15 to 0.25 Gy. There are substantive questions about relations between cognitive and reflex measures and differences between the nature of mental retardation in humans as compared to functional or behavioral changes in experimental animals.

In both animals and humans, evidence suggests it is possible to produce primary neural cytotoxic effects, but precise dose-effect relationships have not been determined for individual types of neurons. As with most cell populations, adequate doses to mitotically active cells lead to DNA degradation and lethality. Because the stage of cell cycle at irradiation and whether the cells are pre- or postmitotic are related to time of migration, the nature and magnitude of effects seem to differ with their temporal relationship to these processes. Secondary effects on neuroblasts may be mediated through nonlethal DNA/RNA modification via indirect alteration of the cellular membranes and/or proteins. There is also a possibility of induction of secondary effects in the developing brain by nontraditional mechanisms, but there are no reasons to expect differences among species. These types of processes may include neuronal events that depend on the integrity of other cells and cell populations, such as guidance of migration and localization, as well as the role of proper synaptogenesis in survival. Changing repair and reconstitution capacities during development influence the chronology of cytologic and histologic characteristics and their ultimate functional consequences. The role of factors that modify injury has not been completely defined and requires quantification.

Information from mechanistic studies suggests that CNS lesions are produced by polycytic effects, i.e., ones involving more than a single cell. This phenomenon illustrates the incompleteness of our knowledge of the interaction between low doses of prenatal irradiation and CNS response relative to the processes by which lesions develop. These effects and uncertainties have practical consequences in low-dose extrapolations and in radiation protection practices for pregnant women. They are significant relative to our

understanding of the processes by which the CNS, or other developing organs, may respond to insult by radiation or other agents.

There are modern cellular and molecular approaches that have been used for study of CNS development and neurotoxiciology, but they have received relatively little application in defining or understanding the effects of prenatal irradiation. Integrating the perspectives of scientists who have been concerned with the effects of prenatal radiation, particularly in the CNS, with those who are involved in the use of modern concepts and methodologies should provide a basis to reconcile disparate findings and help to identify approaches.

SOCIETAL AND REGULATORY CONSIDERATIONS

Broadly unified societal and regulatory concerns were identified and have been summarized under the following five general categories:

1. There is a need for improved risk estimates for effects produced by intrauterine radiation exposures, exemplified here by the function of the postnatal nervous system. From the regulatory standpoint, accurate and realistic risk estimates are important for setting exposure limits that will protect populations, but the limits should avoid undue economic costs and receive acceptance by society. From a broader perspective, rational limits are important to physicians and other health professionals who are involved in the management of pregnant women who may be occupationally or medically exposed.

2. Information needed to improve risk estimates includes better definition of the nature of limiting effects, whether there is a threshold, and more precise knowledge of pertinent dose-response relationships. An important aspect is knowing what levels of risk society would consider acceptable relative to benefit.

3. To maximize knowledge, all possible information should be derived from studies of the Japanese survivors. A primary need is to define how best to use available resources to obtain the greatest amount of information, preserved specimens, additional contemporary data, and requisite statistical analyses.

4. Many issues posed by the Japanese data might be clarified by evaluation of other human populations. Possibilities include obtaining ancillary clinical information from

situations involving medical exposure of prenatal or neonatal populations, as well as those which were monitored following internal and surface radionuclide deposition. The usual situation has been that studies of people have identified phenomena that were subsequently investigated in animals, but experiments might suggest additional measurements or approaches that should be applied to human populations.

5. Additional information is needed to evaluate and understand the impact of other factors that might affect response. These factors include responses to fractionated doses or exposure at low dose rates. Because they could result in increased endpoint sensitivity, exacerbate the injury sequence, or interfere with repair and of injury, it is important to know if prenatal irradiation altered responses to maternal insults such as drug or alcohol consumption and decreased gestational nutrition. Other neuropathophysiologic deficits and relationships require evaluation; these include functional changes that become more marked during aging.

RECOMMENDED AREAS FOR FURTHER RESEARCH

Several recommendations and integrated approaches for research were considered for understanding details of known effects and addressing disparities and uncertainties. Salient recommendations are summarized below. Generalities relative to design features and potential approaches for such investigations are indicated after the recommendations.

1. Studies to obtain information about lifetime effects in prenatally exposed Japanese survivors should continue. They should include definition of disorders, deficits, and their correlations with exposure and dose. Continued surveillance and implementation of assays to detect delayed effects are important. Ancillary investigations are needed to evaluate the role of interactions of radiogenic damage with that from other insults. These studies require close ties between the experimentalist and epidemiologist to carefully design experimental studies.

2. Similar investigations of early postnatal and lifetime CNS effects in other exposed human populations are desirable. These should also include populations that were exposed in the early postnatal period to external radiation or via radionuclides with its unique and phenomenological aspects.

3. There is a continuing need for investigations in experimental animals to quantitate interactions with other potential developmentally toxic agents and events. Establishing such a database is necessary for extrapolations to establish risk to humans. Studies that use nonhuman primates might yield data that would provide links between results from studies of human populations and experimental animals.

4. Additional approaches are needed to facilitate obtaining and using information to establish dose-response relationships and extrapolations. Mechanistic studies were considered to provide steps toward a theoretical basis for using empirical data for extrapolations. Studies are needed to provide parallel experimental bases for using animal data for predictions in women and for extrapolating from acute to chronic and from high-dose to low-dose exposures.

5. Mechanistic studies should be undertaken in which molecular biology and neurotoxicology concepts, approaches, and techniques are integrated into collaborative investigations with other disciplines. Such investigations could provide opportunities for understanding processes and defining an integration of mechanisms and effect. This knowledge should assist in making decisions about the shape of dose-response relationships; it would also identify any possibile thresholds and their magnitude.

Design Features and Potential Approaches to Investigation

Design of Clinical and Epidemiologic Studies

Neurologic examinations should be recorded on videotape for consultation with other neurologists and for archival purposes should additional analytical procedures become available. Examinations should begin at the earliest possible age, and neurologic evaluations later in life should be performed to detect acquired or premature disorders or deficits. Endpoints should include basic movements, behavioral assays, evoked potentials, and noninvasive electronic parameters selected from among EEGs, ECGs, electromyograms, and externally stimulated nerve conductance measures. Sensory monitoring should include visual fields, responses to photostimulation, odor identification, and measurements that would quantitate shifts in curves of graded responses to aid in detecting subtle changes.

There was corroboration of ongoing and planned efforts to collect and store accessible body tissues such as blood cells and serum, sperm, and hair, as well as surgical specimens, and to immortalize white cells for later DNA studies. Magnetic resonance images should be continued; they should be correlated with clinically detectable neurologic changes, and their association with seizures and morphology established when possible. Multiple regression analyses should be applied to past and future data.

Correlations and Design of Animal Studies

It seems unlikely that a theoretically sound basis can be established to encompass all relationships between developmental stage, dose rate and dose, and resulting effects. Information available to toxicologists might facilitate interpretation of epidemiologic studies at lower doses, and heightened communication between experimentalists and epidemiologists was encouraged. The consensus was that (1) individual problems required separation into components, (2) understanding of underlying mechanisms was needed for valid interpretations, (3) collaborations would be important, (4) efforts should be made to overcome primarily semantic differences, and (5) temporary operational agreements should be made to circumvent limitations of unknown theory.

Laboratory animal studies are needed to determine interactions between other insults and prenatal radiation, the role of dosing regimens, and other phenomena such as altered susceptibility to subsequent postnatal insults in surviving populations. The likelihood of producing morphologic lesions in experimental rodents and in humans is similar; however, the relationships to functional endpoints are less sure, so there may be a need for studies of other species. Suggestions centered around nonhuman primates, for which there are batteries of tests and measures corresponding to those used for humans; the crab-eating macaque was considered a likely surrogate species.

Molecular Neurotoxicology for Integrating Mechanisms and Effects

Collaborative approaches should be comprehensive. The efforts of molecular biologists should be integrated into research teams that include investigators in the other fields. A wide range of questions about developmental radiobiology could be investigated in this manner, including determination of reasons for altered functions ranging from cell division and defects

of migration to the associated cell-to-cell interactions. Generalizations about mechanisms could also be tested. Additional approaches include the study of molecular lesions in transgenic animals and their effect on the response of neurons to ionizing radiation and alterations in gene expression.

Molecular neurobiology offers opportunities to expand understanding of recognized processes that may be involved in the genesis of CNS developmental deficits by prenatal irradiation. These include (1) migration, (2) cell-cell interactions, (3) gene expression, (4) cell division, and (5) cell death. To understand such phenomena in terms of molecular biologic changes, it is necessary to understand the role of genes and alterations of their expression in the response of neural cells to ionizing radiation. Before their terminal division, this might be examined in terms of secretion of surface proteins, extracellular matrix (ECM) composition, adhesion, and the roles of these processes in achieving normal cytoarchitecture. For postmitotic cells, it is necessary to identify other relevant responses. The functions of glial cells, which can also proliferate, must be considered in the overall phenomena.

A pharmacological approach, in which protective or sensitizing agents are used in combination with radiation to modify terminal response, might provide useful information. Chemical perturbations could be introduced for selectively testing mechanistic hypotheses and to provide information for extrapolating from rodents to primate species to humans. These are relevant considerations for risk estimates and assessments.

INTRODUCTION

Dr. David Smith, Director of the Health Effects Research Division of the Office of Health and Environmental Research (OHER), introduced the workshop by presenting OHER's perspectives on the problem that led to encourage and support the Workshop. He indicated that recognition of the scope of the problem and concern with its resolution are shared by Dr. David Galas, Director of OHER, and other responsible persons within the U.S. Department of Energy (DOE). He commented that awareness of the societal importance of nervous system disorders extends to Dr. Allan Bromley, Director of the Office of Science and Technology Policy (OSTP), and noted that OSTP's Subcommittee on Brain and Behavioral Sciences was preparing an overview to celebrate the Decade of the Brain (1990-2000). The importance of the radiation question is further evidenced by the high priority assigned by the Health and Environment Research Advisory Committee to the Office of Energy Research to the need for further exploration of health risk and the need for simple and model systems for studies of neuronal biology and interactions. Dr. Smith concluded by noting the growing recognition that we are now entering into a new age of biological investigation, with additional research technologies applicable to prenatal irradiation of the central nervous system (CNS).

CLINICAL AND EPIDEMIOLOGIC PERSPECTIVES

HISTORICAL PERSPECTIVES

The initial session was designed to provide background information relating to exposure of the human conceptus. Dr. James Yamazaki of UCLA, who was in the initial contingent of physicians in postwar Nagasaki, presented the scientific introduction and historical perspectives. He began by noting the sequence of past meetings and reports dealing with the overall problem, especially published papers and reviews that collated results of early work involving clinical investigations, which in turn led to extensive laboratory investigation. These studies had documented reduced stature and head circumference, mental retardation, and ocular defects, but interest had largely lain dormant. Dr. Josef Warkany's tutelage of the clinical cadre before its departure for Japan caused them to look for possible effects on the CNS.

These effects a e evident in the Atomic Bomb Casualty Commission (ABCC) reports that describe results of early evaluations of prenatally irradiated Japanese children at Nagasaki. Dr. Yamazaki noted that they had identified 98 surviving women who had been pregnant within 2000 m of hypocenter. Of these, 30 displayed radiation sickness or signs (epilation, oropharyngeal lesions, petechiae, and/or purpura). Seven (23%) of these pregnancies resulted in fetal deaths (3 abortions; 4 stillbirths), and there were 6 women (20%) whose children died as neonates or infants. In contrast, 8.8% fetal mortality and 6.2% neonatal/infant mortality occurred among the 68 pregnant women in this same area who did not display radiation signs. For comparison, there was a combined 6.2% incidence of fetal and infant mortality in an age-matched group of 113 age-matched pregnant women who were 4000 to 5000 m from hypocenter.

One of the 17 surviving children from the group with major radiation signs died at 2.5 years of age. Four of the remaining 16, all of whose mothers had been 850 to 1150 m from hypocenter, displayed varying degrees of mental retardation and retarded physical development. Mean head circumference was significantly reduced in children born to the group with major signs as compared to the groups without major signs and the controls; the four smallest head circumferences were measured in mentally retarded children. Despite the confounding of patterns by a lack of dosimetry and the presence of other

factors such as trauma, burns, hemorrhage, infection, and malnutrition, the investigators interpreted the evidence to suggest that the magnitude of the irradiation determined pregnancy outcome and developmental status of the offspring.

The initial study group in Hiroshima consisted of the children surviving to 4.5 years of age following birth to mothers who were exposed during their first 20 weeks of gestation. This group consisted of 205 children whose mothers had been within the Hiroshima city limits at detonation; it included 11 women who were within 1200 m of hypocenter, 7 of whom had definite signs of exposure. Five of the children born to these women with radiation signs had mental and physical retardation. None of the 194 children from mothers exposed at greater distances were microcephalic, but 7 children born to the 11 women in the group less than 1200 m from the center were mentally retarded and had reduced head circumference. Subsequent reports on developmental effects determined through follow-on studies of 169 living survivors whose mothers were within 2200 m of hypocenter indicated that the head circumference of 33 children exposed between the 7th and 15th week of gestation was 2 or more standard deviations below the average for children of comparable age and sex. As diagnosed by clinical opinion, poor school performance, and unsatisfactory performance on psychometric tests, 15 of these 33 were judged to be mentally retarded. The incidence of mental retardation and microcephaly also decreased with distance from hypocenter, and these findings were unchanged by an additional evaluation when the children had reached 17 years of age. None of the other observed defects were found to correlate with distance from hypocenter, although radiation dose estimates were not available.

Subsequently, radiation doses (T65D) were estimated on the basis of distance and shielding configuration, sample sizes were enlarged, and individual doses were estimated for most of the pregnant women. Calculations on this basis disclosed that the incidence of small head circumference increased with dose among children born to women exposed before the 18th week of pregnancy. The lowest dose range that produced this defect was 10 to 19 rad in Hiroshima, but no effect was detected below 150 rad in Nagasaki. The small head circumference was often accompanied by mental retardation among children in both cities. Production of these dual defects usually occurred at doses of 150 rad

and above, which is in general agreement with a minimum of 250 R that had been estimated from an analysis of cases reported in the literature. These reports include several descriptions of findings at autopsy of abortuses from women who had received high radiation doses consequent to radiation therapy during pregnancy or during attempts at radiogenic abortion, and provide most of our information on radiogenic morphologic changes in the human conceptus.

EPIDEMIOLOGY AND CURRENT RERF ACTIVITIES

Dr. William J. Schull, of the University of Texas Health Sciences University in Houston, was among the early participants in the Genetics Group of the ABCC. He and his colleagues subsequently reanalyzed the data and epidemiologic inferences relating to CNS function in the prenatally irradiated Japanese populations. It was especially appropriate that he was able to participate while enroute to Japan to assume his duties as a Permanent Director of the Radiation Effects Research Foundation (RERF).

Dr. Schull described the evolution of epidemiologic evaluations using population statistics based on dose estimates and the sequence of observations and conclusions from follow-on studies. He indicated that he would confine his discussion primarily to events in Hiroshima and Nagasaki, and noted that opportunities remained here for expanding our information base. He expressed hopes that the Workshop would provide him with additional investigational approaches by way of expanded batteries of neurologic measures and procedures that could be used in Japan. For emphasis, he mentioned that a newsletter item indicated that 90% of what we know about the brain has been learned in the last 10 years, and suggested that, because as most of our information about prenatal radiation injury precedes this era, it has not been fully exploited relative to mechanisms.

He reinforced the observation that unknown dose was a problem with early analyses. It was not until 1965 that first dose estimates (T65D) were made from kerma in houses, and that most absorbed doses were less because of shielding. It was not until 1978 that the first fetal dose calculations were prepared. The revised approaches used for the recent (TS 86) dosimetry provided dose to uterus, and fetal doses are currently being calculated. The original study population, denoted PE 86, consisted of 2200 individuals and

did not account for those who terminated pregnancy outside of Hiroshima and Nagasaki. Most statistical analyses and results that he described had been performed on a revised PE 86, denoted as the clinical sample, which contains 1600 members of the original sample.

This population does not include children of women who were between 2000 and 2900 m, a set that will be reconsidered because it is now recognized that all doses may not be trivial at 2000 m. Between 400 and 600 members of the clinical sample are now followed on a 2-year cycle, the reduction being due to migration from the city; however, diligent effort might increase this to 600 to 800. Checks on the completeness of the census of affected persons by comparisons with the census of other groups detected additional cases. These cases have not been included in statistical analyses because the denominator is not known, although there is interest in these cases for study of mechanisms. An additional source of uncertainty is that some women probably delivered early or prematurely, thus overestimating gestation time at exposure as calculated by counting back from birth date.

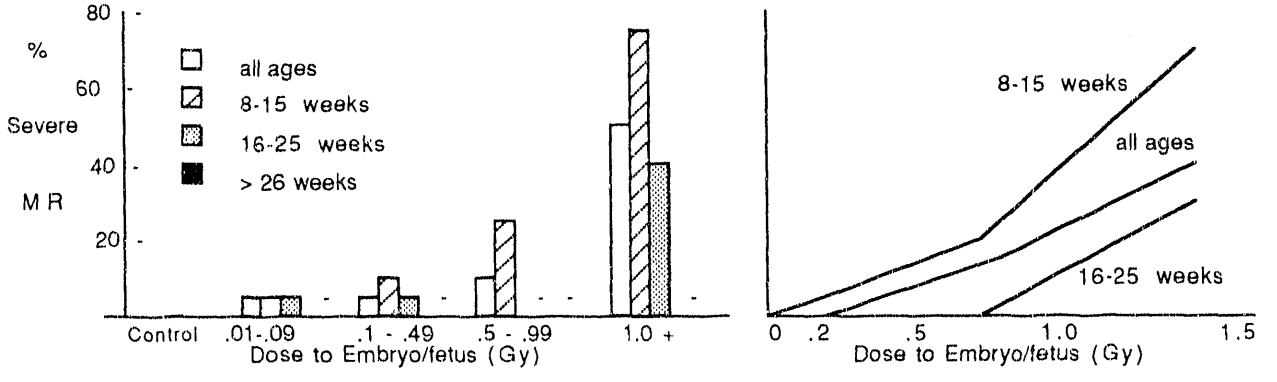
Schull and colleagues devoted their primary statistical considerations to five affected functional measures: First, and most important, was the incidence of persons with mental retardation and its subset classification of severe, which were the initial CNS measures determined in these populations. Second, about one-third of the offspring had been selected for scoring of school performance to examine its correlations with psychometric testing during childhood. Third, the children had been evaluated using the Koga intelligence test, which was employed in the school system at that time. Among those who were mentally retarded, the highest IQ score was 64, most scores were in the mid-50s, and some children were untestable. The fourth measure was incidence of unprovoked seizures; the fifth was motor skills, such as grasp strength and repetitive test performance. The extent to which these measures are independent or are manifestations of common lesions is not certain at this time.

Dr. Schull indicated that he would not dwell on questions of threshold or the shape of the dose-response relationship because it was necessary to know more about fundamental interactions before making final decisions. The results of statistical comparisons and inferences from considerations of embryological

stages of brain development had guided selection of the developmental-stage groupings indicated in the column graphs in Figure 1A andB. Some of the data sets he used for illustrations were the same as those he has used in previous presentations and publications and which were the basis for analyses, evaluations, and calculation of risk estimates by advisory groups. Many measures for the clinical sample tended to follow the general patterns for severe mental retardation, which are redrawn here in simplified form to typify the several illustrations he presented.

Analyses of some of the incidences indicate higher sensitivity in the 16- to 25-week group than in the overall population, but the most striking elevation of dose-response effects was for those exposed at 8 to 15 weeks. A linear fit was used for illustration to facilitate comparisons at a fixed dose, 1 Gy in this case. Incidence for all ages was 28%, but was about 20% and 40%, respectively, in the indicated two groups.

He illustrated some tentative mechanistic inferences concerning relationships between morphology and function that were made from the limited anatomic information that they had obtained. He expressed hopes that the database would expand with time. As of 1986, four postmortem examinations had been



A. Incidence in dose groups within age cohorts

B. Effect/dose in three age cohorts

FIGURE 1. Relationships between severe mental retardation, embryo/fetal dose, and age

performed. Two of these were people who were not mentally retarded, and their brains were of normal weight and structure. The other two had been mentally retarded; one was a female who received about 0.01 Gy after 30 weeks of gestation. Her brain was smaller than normal but neuropathologic lesions were not detected. The other case was a male who died at 16 years of age with fulminating encephalitis. He had been exposed at 12 weeks of gestation to a dose in excess of 1 Gy. He had several problems throughout his life, including cataracts, bilateral retinal fibroplasia, and bizarre sexual behavior. Autopsy findings included a reduced brain weight of 800 g, massive areas of ectopia around both ventricles, bilateral hypoplastic adrenals. One of his testicles was small, the other was undescended.

Dr. Schull had been intrigued by similarities of this condition to Kallmann's syndrome, which involves hypogonadism and hypogonadtrophism and is a common cause for males to enter infertility clinics. Kallmann's syndrome is characterized by anosmia. The researchers were unable to determine whether this boy had a deficient sense of smell. Within the last few years there have been two descriptions of embryological changes associated with this syndrome, in particular abnormal development of luteinizing hormone releasing hormone (LHRH) neuronal cells. Normally, small numbers of these ciliated cells originate in the olfactory placodes and migrate into the cerebrum at about 12 to 14 weeks of gestation. In one autopsy (on a 19-week embryo) these cells had accumulated locally at the interface, suggesting that some essential chemical signal might be absent. A mouse surrogate for this disorder is now available, and may provide a basis for study.

These observations led Dr. Schull's group to further explore correlations with structural abnormalities, and they obtained magnetic resonance images (MRI) from five exposed individuals who were selected because they were mentally retarded and microcephalic. It was noted that MRIs of mentally retarded individuals who have not been irradiated show areas of ectopia, although incidence values are not available, nor is pattern known in functionally normal individuals.

The images from two males who had been exposed to about 0.7 Gy at 8 to 9 weeks of gestation showed lumpy aggregates of cells in regions of gray matter where they normally appear as thin lines, in addition to periventricular

ectopia. Mild microgyria and abnormalities of the corpus callosum were observed in the most severely affected of the two females, who had been exposed to 1.2 Gy at 12 to 13 weeks. The cisterna magnum was markedly enlarged, and the corpus callosum was atypical, perhaps through compression. One female also had bilateral hip dislocation; this could be a radiation effect because exposure coincided with the time the acetabulum forms. The fifth MRI case was exposed at 15 weeks; the brain was small, but no lesions were detected.

In response to questions during discussion, Dr. Schull indicated that neurologic examinations had not been helpful beyond establishing the original diagnosis, but EEGs had not been performed in any of these cases. Further discussion included suggestions that a cerebellar workup might be useful, comments to the effect that the cerebellum could be removed in the young child and regenerate normally, that this ability to repair decreased during development, and indications that MRI and quantification of responses might be capable of detecting function abnormalities in the absence of overt clinical impairment.

OTHER FACTORS INFLUENCING CNS RESPONSES

Dr. Yamazaki then presented a clinical perspective that considered health and emotional stresses that were experienced by the exposed population, and served to introduce uncertainties in interpretation. He noted that there were several concomitant and subsequent circumstances that might be expected to have adverse effects on prenatal development, or to synergize with radiation or other insults. These are:

> Malnutrition - An effective naval blockade at the time the bombs were dropped reduced importation of food from the mainland; aerial bombardment also impeded food distribution. There was clear evidence of impaired nutrition: beriberi among factory workers, anemia, and lowered blood protein levels, as well as inanition associated with food having unpleasant odors. Clinical studies following World War II demonstrated an effect of nutrition on birth weight, although it did not increase malformation incidence, as in experimental animal studies. Related nutritional factors such as anorexia and diarrhea were superimposed among the symptomatic women, which could exacerbate the intrauterine growth retardation, low birth weights, and the attendant myriad of

problems of prematurity that included bleeding into the periventricular areas and its profound neurologic sequela.

Blast and Heat - The mentally retarded children who were irradiated constitute a unique group in several respects because their mothers underwent several additional traumas during their pregnancy. As with radiation, blast and heat decreased with distance from hypocenter, but the insults and their effects were still substantial at 2000 m. Acute effects in this range included extensive blast-related mortality as well as both flash and thermal burns in survivors. Heat intensities were sufficient to blister paint and scorch wooden surfaces at distances well beyond 2000 m.

Maternal Irradiation - Published studies of experimental rodents had demonstrated that effects of prenatal radiation were produced by direct effects on the embryo and were independent of the maternal exposure. Nonradiation lesions from the bomb detonation were severe, however, and there were multiple sources of generalized effects in the pregnant Japanese women in the highest radiation dose groups. We must therefore accept the possibility that there were interactions with the effects of the several specific insults that were experienced concurrently with the irradiation.

Infection - No specific neurotoxic viral or bacterial epidemics that might be expected to exacerbate the radiation effect on the developing brain had been identified in either city. There was a typhoid fever outbreak in Hiroshima subsequent to a typhoon that occurred a few weeks after bombing. Many mothers reported recurrent febrile illnesses during their pregnancies, especially those who had evidenced radiation symptoms. The effects of these febrile episodes may warrant consideration because of more recently reported information about hyperthermia-induced teratogenesis, its possible interaction with ionizing radiation exposure, and interactive mechanisms of effect.

Psychosocial Impact - The scientific literature does not fully consider psychosocial impacts on the affected survivors and their families, which may exacerbate lifetime detriments. This problem is exemplified by the existence and activities of the Kinikokai organization, loosely translated as the Mushroom Cloud Auxiliary, which is composed of mothers with a retarded or microcephalic child. A wide variety of discriminatory actions were directed toward these Pika (Japanese for flash) children and their siblings. They were subjected to social stigmata, and these were superimposed on their mental retardation and their increased incidence of other defects and illnesses. All these factors combined to result in detrimental economic consequences, but there is no way of quantifying the interactions and contributions of these confounding conditions.

In response to questions, Dr. Yamazaki indicated there was no way of determining if any of these insults, per se, might have affected development.

Moreover, he was unaware of any knowledge as to whether there might be addition or interaction between these insults or between malnutrition and radiation.

RADIATION TERATOGENESIS IN ANIMALS AND HUMANS

Dr. Robert Brent of the Jefferson Medical College and the A.I. duPont Institute combines the perspectives of a practicing clinician, laboratory investigator, and active participant in the public arena. He was asked to introduce concepts of embryofetal toxicity and teratogenesis, in addition to addressing correlations that might be obtained through evaluations of additional populations.

Dr. Brent pointed out that a significant part of the societal concern about radiation results from misinformation of professionals and the public, and he used testimony in a recent lawsuit as an illustration. The case involved a 4.5-month-old infant who entered the hospital for respiratory problems and developed a gastrointestinal infection with complications that resulted in a comatose state for 11 days. Head size and intelligence did not increase following release from the hospital, and microcephaly and mental retardation were evident at 4 years of age, when articles in the popular press stimulated litigation. Even though the child had normal head size at birth and upon entry into the hospital, two expert witnesses testified that the microcephaly was due to gestational exposure to diagnostic x rays.

Dr. Brent cited early rodent studies in his laboratory which demonstrated that radiation-induced intrauterine mortality and teratisms are due to direct effects on the embryo and are not mediated by irradiation of the mother. He said that animal models were important for effects from external radiations and were more appropriate surrogates than they were with chemical teratogens because as metabolic factors and placental transfer differences do not play a role in exposure of the conceptus. He also thought that quantitative results could be extrapolated more readily. Species differences were considered to be of even less importance when chronological times were properly scaled to developmental times and stages. This is exemplified by the fact that the period of major organogenesis constitutes 25% of rat gestation as compared to 5% to 10% of human gestation. This shorter period of sensitivity for most teratogenic effects, other than the CNS and a few other organs, may explain the failure to detect abnormalities in epidemiologic studies, in addition to the exposure doses being too high or too low.

In the rat, the transition to sensitivity to teratogenesis is gradual, rather than abrupt, and occurs over several hours on the 8th day of gestation. Radiation sensitivity for malformations of most systems in the first trimester of humans starts on the 17th to 18th day and extends through the 36th day. The second trimester continues to be important for effects on some systems, including gonads, sex organs, kidney, uterus, and the palate, while brain sensitivity extends through these periods, through the third trimester, and into the postnatal period.

Dr. Brent believed that cellular studies could not directly answer all questions regarding incidences and that animal experiments were required for defining dose-response relationships. For understanding, it is necessary to make a distinction between stochastic and nonstochastic effects. Theory indicates that only a single cell must be affected for stochastic effects (mutagenesis and carcinogenesis), but incidence does not reach 100% because of cell killing. Teratogenesis is nonstochastic, because many cells must be involved in providing the initiating event; incidence and severity increase with dose, and incidence can reach 100%. As a consequence of repair and recuperation, protraction or fractionation decreases the impact of radiation on effects ranging from general malformation production to reduction of brain layer thickness. He had combined results from his laboratory exposure of rats after 9 dg, the most sensitive stage for teratogenesis. Incidence as a function of dose showed a no-detected-effect (threshold) level at about 0.1 to 0.2 Gy.

He presented several lines of evidence that explained the later sensitive period for persistent brain effects as being related to cell migration and to the progressive loss of the ability to replace cells and recover normal structure. To illustrate approaches to separating components of the syndrome, however, he noted that children with hydrocephalus can have a good prognosis if their brain is protected from secondary effects. From the standpoint of published case reports and reviews of the older literature, among fetuses or children from pregnant women who received radiation therapy at the 2.5-Gy level or above, the CNS was affected in all cases; malformation of other structures did not occur without CNS involvement.

He indicated that estimates of risk for medically indicated radiation exposures in NCRP Report No. 54 would serve as a summary of quantitative conclusions. This report, as well as others, discussed various minimal exposures that had been shown to produce effects, but did not conclude whether there is a threshold. Report No. 54 noted that doses "... below 5 rad to the human embryo-fetus are considered by many to represent an acceptable risk when compared to the potential medical benefits..." The NCRP report distinguishes, however, the status of the embryo-fetus in occupational as contrasted to medical environments.

Dr. Brent described three main categories of epidemiology. First, the cohort study parallels comparisons made among groups of experimental animals. This type of study requires identical characteristics in control and treated populations to detect differences in response. This qualification is not usually met in epidemiologic studies because of uncontrollable differences, and adequate numbers of subjects are rarely available. This type of study is the least sensitive, but it has the lowest rate of false-positive findings.

The second type is the case control study, which involves looking for a difference in exposure between the normal or unaffected group and the affected group that displays the pathology of interest. These are the most common types of study because of their lower cost and need for fewer subjects. They have both greater sensitivities and higher error rates than cohort studies.

The third category, the cluster, is based on observation of relationships by alert physicians or scientists. It is most sensitive, and initially identified essentially all human teratogens, but is most prone to error. Experimental animal studies have not been very useful for predicting chemical teratogens. They are improving, however, as pharmacokinetics are increasingly employed, allowing use of embryo cultures.

Dr. Brent observed that we are usually critical when evaluating papers in our own area of expertise, but we may accept weak results and unsubstantiated conclusions in reports outside our field. He thinks that there are five criteria by which the conclusions of epidemiologic studies should be evaluated, and he used these to consider evidence relating to the question of low-dose radiation and mental retardation.

The first criterion, consistency of studies, is difficult to evaluate because only few studies have been performed. The ABCC/RERF database contains few cases in the low-dose range, which makes internal consistency impossible to determine. There are no independent studies that confirm the conclusions of the statistical interpretations, but, on the other hand, there are none that refute them. The few reported integrated reviews of clinical findings are based on small groups, composed mainly of offspring of women who had received large doses for radiotherapy, and the results do not bear on the question.

It is always possible that a positive finding had been obtained by chance, and independent investigations are needed to rule out or support this possibility. There are two potential sources of future results that could provide some of the needed data and interpretations relative to low doses. One is a 1000-patient population, most exposed via pelvimetry, which investigators at the University of Chicago have proposed to study. Dr. Brent hopes for an evaluation of data from the Collaborative Perinatal Project of the NIH; this population involves 50,000 subjects, of whom 11,500 received low-dose radiation from abdominal exposures of their mothers. Subsequent discussion questioned whether these group sizes were adequate to warrant evaluation, but no conclusions were reached.

The second consideration, secular trend analysis, has limited applicability to the question of mental retardation of offspring, despite the large number of people who have received low-dose radiation during pregnancy. In particular, diagnosis cannot be made at birth, and there are numerous etiologies. The third criterion, response patterns in animal models, is a basis for judgment and has provided some confirmation of similar stage-dependent differences in sensitivity and clear evidence for the existence of CNS responsiveness. The fourth criterion, the existence of a dose-response relationship for several endpoints, consistently displays a no-effect range in animal studies, and has not detected relevant effects at the lowest doses.

The fifth and final consideration is scientific or biological plausibility. It requires making a decision about whether the dose-response relation is linear or not to determine whether to accept that very low doses could cause severe mental retardation. The need for damage to several cells for

detectable effects and the consistent lack of such overt change below about 10 rad lead him to believe there is a threshold. He stated that even if one accepted that there was linearity, no threshold, and the calculated risk for IQ reduction was 30 to 40 per Gy, straight-line extrapolation to 1 rad or 0.01 Gy would yield a decrease in IQ of 0.3 to 0.4. He considers that this is not consistent with mental retardation and would require invoking an unknown mechanism that pertained at low dose.

In response to a question, Dr. Brent indicated that he believes that malnutrition could interact with radiation to quantitatively increase the response. However, he would not expect it to yield a qualitative difference. As a primary mechanism, there could be a diminished rate or extent of repair in the absence of adequate nutrition.

EXPERIMENTAL ANIMAL STUDIES

ABNORMAL MORPHOLOGY-FUNCTION RELATIONSHIPS

Dr. Stata Norton of the University of Kansas reviewed our knowledge of the behavioral and functional deficits detectable after prenatal irradiation and their relationships to morphologic effects. She illustrated many of the points with research results from her laboratory as well as from published collaborative efforts with others to interpret the overall information base.

Individual components of the CNS and sensory organs have different gestational periods for maximum radiosensitivity, extending well into postnatal life of the rat. There are two periods of maximum sensitivity that result in learning disabilities in rats. The first reaches a peak around gestational day (gd) 15, and is presumed to represent cerebral cortical damage; the second peak, around postnatal day 5, may involve hippocampal damage. Following 1.0 Gy on gd 11, 13, 15, or 17, decrease of body weight shows a tighter "window" of sensitivity than does the decrease in cerebral cortex thickness, which involves both neuron number and synaptic connections with fiber ingrowth. The layers of the cerebral cortex are formed over a several-day period, starting on gd 11; the peak effect on the lower layers is around gd 15, while the upper layers (I or III) are maximally affected on gd 17. For comparison of rats and humans, based on corresponding somite stage and formation of the cortical plate, gd 15 to 16 in rats correspond to gestational days 45 to 50 in humans. Studies have correlated exposure at different developmental stages with subsequent behavioral/functional effects.

A brief general description and diagrammatic representation of corticogenesis is included in the presentation by Dr. Konermann, which follows. As described by Dr. Norton, however, the cortical mantle consists of a germinal matrix, subventricular zone, intermediate zone, and cortical plate on gd 16. When the cortex is examined for dead cells at 24 hours after exposure to 1 Gy on gd 15, the intermediate zone and cortical plate show large and significant dose-response relationships for increased numbers of pyknotic cells over the dose range from 0.25 to 1.0 Gy. The cerebral cortex has considerable ability to repair reduced cell count at 1.0 Gy and below, but the behavioral damage is permanent and may even become progressively worse as the

matures. Three hours after exposure to 0.5 Gy, most dead cells are in the subventricular zone, and their number drops progressively. Concurrently, the percent in the intermediate zone and cortical plate increases through 24 hours, suggesting that dead and dying cells are still able to migrate. Macrophages migrate in by 6 hours and phagocytosis has decreased the total number of dead cells by 24 hours.

Several functional defects exhibit similar dose-response curves, and as with pyknosis, doses from 0.25 to 1.0 Gy to rat fetuses on gd 15 may affect several postnatal reflexes, as well as body weight. Numerous investigators have studied relationships between gestational age at exposure and effect on behaviors, including surface righting, negative geotaxis, reflex suspension, open field activity, and spatial maze or continuous corridor activity. In general, functional tests of motor ability in the preweaning period are affected only by higher doses ($\geq$1.5 Gy), except at gd 17. Activity in the open field has been studied after exposure to doses from 0.25 to 3.0 Gy and at gestational times from day 6 to 22. Both increases and decreases in activity have been reported, and generalizations are uncertain. All tests showed varying degrees of dose responsiveness, and the lowest dose that has been found to be effective is 0.25 Gy, while 0.5 Gy at any time in this range affects activity.

Decrease in body weight from gestational irradiation is most marked during the preweaning period, and weight may show some recovery during maturation after doses below 1.0 Gy. The various tests of cognitive performance reported in the literature, however, do not indicate improvement with maturation. On the contrary, doses less than 1.0 Gy sometimes produce no behavioral effect in early tests, but alterations are detectable if the rats are tested when they are older.

An additional test that has been used to study the interaction of postnatal age and a single gestational radiation exposure examines the possibility of progressive damage in terms of multiple components of behavior. A control and exposed rat are placed in a double cage, and behavior is recorded by time-lapse video photography; body position is converted to a computerized description of body shape and interpreted as a behavioral act. Duration of each act (in seconds) and interval between occurrences (runs) of the same act are

recorded. When these behaviors are compared at 1 month (weanling) and 3 months (mature), there are significant differences between irradiated and controls at both times. A decrease in duration and decreased intervals between exploratory acts at 3 months, compared to 1 month, indicate that the rats are becoming more hyperactive with maturation. This concept has importance for human behavior since other studies of hippocampal irradiation in rats also have reported progressive worsening of behavioral measurements.

Studies of the relationships between functional and morphologic damage to the CNS have included changes in the cerebral cortex and in other sites (e.g., hippocampus and cerebellum) in which morphologic effects are also recognized as affecting behavior. Measurement of cortical thickness in the region of the sensory motor receiving areas were examined for correlations with functional test results. The data were analyzed by multiple regression to determine significant associations of between-animal variation in cortical thickness and performance in functional tests. Computerized photo-analysis was a better predictor of cortical thickness for 3-month-old animals than at 1 month, and there was a further increase in predictability in mature rats. Behavioral acts were generally better predictors than were activity tests, whether determined at 1 month or 3 months of age. The association of behavior and cortical thickness accounted for up to 66% of between-rat variability in controls; however, there was a poorer, but significant, association in irradiated rats. This result suggests a causal relationship between morphologic damage of the cerebral cortex and its function.

Dr. Norton's opinion is that there is no single "best" behavioral and functional test, and that the several tests measure different components of behavior. Some tests are more broadly based in terms of function, and the intended use of the data should dictate the choice. Correlations still need to be explored in greater detail, and long-term consequences must be understood in terms of specific functions rather than global interpretations. All details of correlations between brain morphology and behavior are still not resolved, and the lowest effective dose for many tests is not known; determination requires a study where the "gestational window" is known because of interactions between dose and gestational age.

Dr. Norton also addressed questions concerning the shape of the dose-response curve in the range below the lowest dose that results in a significant effect; in other words, is there a threshold? She considered that the lowest effective dose was about 0.25 Gy, but dose-response curves at low doses cannot be predicted. Further investigation is needed to establish correlations between cortical thickness, diminished neuron density, diminished synaptic connection, and diminished function. An unanswered question that arose for discussion here, after other presentations, and during final discussions was: Do pyknotic neurons migrate?

ALTERED NEURONAL ALIGNMENT

Dr. Gerhard Konermann of the Institute of Biophysics and Radiation Biology of the University of Freiburg, Germany, was asked to address low dose effects on cellular migration and its patterns. In particular, he was asked to illustrate consequences in terms of altered alignment and connectivity of neurons and fibers. These involve newer techniques that are used in his laboratory for quantification of patterns in histologic sections by computer-assisted image processing and analyses.

He agreed that predominating endpoints were related to stage of gestation at exposure in all mammalian species. The early postimplantation period, 6 to 9 days in rodents, still involves many all-or-none responses. The induction period, which may extend from 10 dg through 18 dg, is the major time of cell formation. However, this period is characterized by a lesser ability to repair, and this ability progressively decreases with time. Irradiation of mice at 13 dg with 0.5 Gy yielded a clear decrease in postnatal myelin density, averaged over all areas; the effect showed a pronounced dose response. Tigroid formation, as determined by staining for Nissel substance, varied among areas. On average, however, the extent of diminution measured in postnatal mice irradiated in the prenatal or early postnatal period decreased as age at exposure increased. Various biochemical measures showed less response; for example, ATP levels had been expected to be a highly sensitive indicator, but were not affected at either early or late times after exposure.

Corticogenesis is a complex process of migration and proliferation through which the several layers of the cerebral cortex are formed, neuronal

processes are laid down, and synaptogenesis occurs. Photomicrographic and diagrammatic representations of the process were used in Dr. Konermann's and other presentations to provide orientation to cortical development and postnatal structure. A simplified composite general diagram (Figure 2) serves to facilitate interpretation of the descriptions in this and several other presentations. Complete detail is not required here, but a synopsis of the explanation that Dr. Konermann presented will be useful for understanding his findings, as well as for some of the other talks.

Autoradiographic studies demonstrate that synthesis takes place in cells of the S zone of the subcortical plate, which then migrate to the periventricular zone while in G_2 where they undergo mitosis. One daughter cell returns to the S zone but the other continues to migrate to become positioned in the cortical plate. Subsequently formed cells migrate through those previously entering the cortical plate to assume a location closer to the surface, so that cells closest to the surface are most recently formed. It is thought

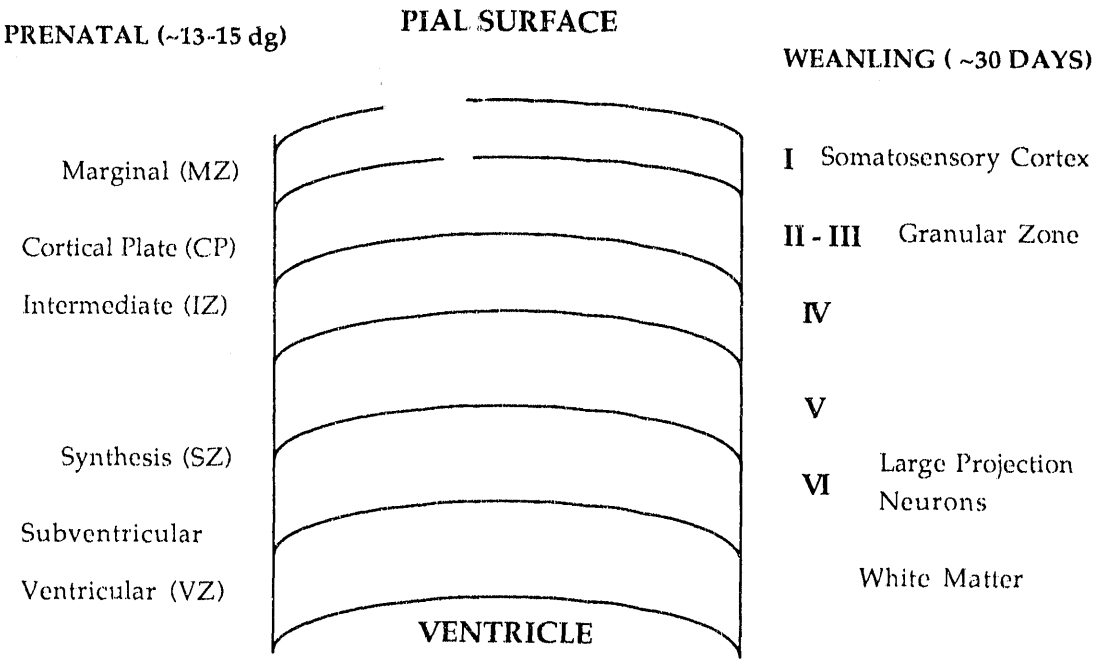


FIGURE 2. Diagrammatic representation of the layers of the prenatal and weanling brain

that the migration pathways are associated with the course of neuronal process formation. It is believed that these processes are under hypothalamic control and operate via some form of contact guidance.

The sequence requires temporal and spatial precision for correct neuronal positioning and synaptogenesis, especially because misplaced cells tend to die. Dr. Konermann also felt it unlikely that these multicellular events would display a linear dose response, or that it could result from alteration of a single cell. After irradiation with 0.75 Gy at 13 dg, a 1-hour pulse labeling with tritiated thymidine at 14 dg yielded autoradiographs showing relatively little decrease in synthesis or cell death in the S- or periventricular zones. Most pyknotic cells were detected in the zones of migration; this contributes to the decreased cellularity and thickness of the cortical plate. Thus, it would be difficult to detect radiogenic cell death against a 15% background of programmed cell death.

A series of photomicrographs of 4- to 6-week postnatal brains demonstrated that prenatal irradiation resulted in progressively severe dose-dependent disruptions of the parallel orientation of neuronal processes in the 5A zone, which contains the large pyramidal cells. Manipulation of histologic sections using a SEIS Zeiss image analysis system provides digitized images for elimination of extraneous characteristics. The processed images were used for quantification of relative fiber parallelism and crossing and provided a value for each section, which is known as the Alignment Quotient. This value showed a dose-dependent decrease for each age of exposure. With exposure at 12 days, there was a 50% reduction in the 0.10- to 0.25-Gy range. Exposures at later times of gestation were progressively less effective, and the threshold range increased. Structural effects were more constant than cellular effects. The corpus callosum seemed to be the most sensitive area of the cerebrum, and the lowest effective dose at which an effect might have been detectable was about 0.05 Gy.

ALTERATION OF NEURONAL DIFFERENTIATION

Dr. Yoshiro Kameyama has made numerous contributions to our understanding of the effects of prenatal irradiation and other insults on brain development

as Director of the Institute of Environmental Medicine of the University of Nagoya, Japan. He was asked to draw upon the research from his laboratory to provide an overview of stage-related effects on the numbers and characteristics of cells of the developing nervous system and to discuss alterations of their differentiation and architectural organization.

To provide a basis for the transitions that would follow, he discussed species and sensitivity differences in developmental patterns as they affect response. He reinforced previous observations and noted that the period of high susceptibility of the developing nervous system extended from the beginning of organogenesis through the postnatal period in all mammalian species. Undifferentiated neural cells are vulnerable to a wide range of environmental agents and are affected by more than two-thirds of the chemicals that have been confirmed as human teratogens.

Effects on the CNS are exacerbated by the progressive loss of neuronal reproductive capacity. There is a prevalence of cerebral histogenetic disorders that are manifested as functional deficits without gross brain malformations. As had been noted, scaling is required when relating susceptibilities for specific effects to developmental stage at exposure. Phylogenetic differences also must be considered in interpretating interactions between the supralimbic lobe, which predominates in humans, and the rhinic-limbic lobes that are more important in rodents.

Mammalian species display generally similar teratologic characteristics, especially those considered as organogenetic. These are characterized by a short, stage-specific sensitive period during early development, and relatively high capacities for restoration. The stage specificity of histogenic changes is less pronounced, displays less capacity for recuperation, and tends to give rise to histologic aberrations and interference with differentiation and growth. The sensitive stages associated with proliferation, migration, and differentiation of ventricular cells begins about 12 days of gestation in mice and 7 to 8 weeks in man, and the process tends to be completed by term in rodents and 16 to 20 weeks in humans, although migration may continue through 25 weeks of gestation. Both sensitivity and the capacity to restore damage decrease throughout this period, yielding marked changes in the resulting patterns of damage. Development of the human telencephalon predominates at

7 to 11 weeks of gestation. Animal data may not pertain for direct quantitation but are useful for determining relative sensitivities and study of mechanism.

Studies involving irradiation of mice with 0.5 or 1.0 Gy on a single day between 10 and 17 dg and evaluation during adulthood resulted in a detectable reduction of cortical thickness due to hypocellularity only after irradiation on day 13. Counts of percentage of pyknotic cells in fetal brains during the 24 hours after irradiation with 0.12 or 0.24 Gy showed peak incidence to be twice as great after exposure at 13 days as on days 10 or 15. Linear dose-response relations for pyknosis were found over the range of 0.03 to 0.48 Gy at these stages. The line fit to the data at the intermediate (13 day) stage indicated that there was a small but significant increase for the lowest (0.03 Gy) dose, while the 0.48-Gy dose produced a 25% incidence of pyknosis.

Dr. Kameyama indicated that impaired migration, which leads to heterotopic gray matter and disorganized architecture of the cortical lamina, may be superimposed onto the cellular deficit. Studies using tritiated thymidine and doses of 0.25 or 1.0 Gy demonstrated that the sensitive period for producing aberrant cortical architecture extended to 15 days of gestation. An altered dendritic branching index for pyramidal neurons in adult mice was significantly reduced by irradiation with 0.25 to 1.0 Gy on 13 or 15 days of gestation.

The mechanisms for these effects are not known. Peak sensitivity for induction of microcephaly in humans seems to be at about 8 to 9 weeks, with an estimated threshold of 0.4 to 0.5 Gy. This corresponds to the beginning of the 8- to 15-week human period of maximum sensitivity for production of mental retardation, for which he suggested a threshold of 0.2 Gy, as well as to the effective periods for most related effects of 13 days in mice and 15 days in rats.

Studies were performed to examine cell death of an apoptotic nature and to decide between delayed or primary damage. Split-dose effects were examined in terms of pyknosis after two 0.12-Gy exposures at a 6-hour interval relative to a single dose. Incidences were almost the same, indicating that acute cell damage by doses in this low range were not repaired in this interval.

Pyknosis incidence began to increase by 2 hours and peaked at 6 to 9 hours, suggesting interphase death. Death of external granular cells of cerebellum, which have similar radiation sensitivities, was completely inhibited by treatment with the protein synthesis inhibitor, cycloheximide, suggesting that the cell death could be regarded as an apoptosis, which is associated with protein synthesis.

In studies to determine explanations for differential sensitivities, Kameyama and colleagues measured the length of G_1, in hours, relative to time of gestation in mice and found a progressive increase: 10 days, 0.1; 13 days, 6; 15 days, 7; 17 days, 14. Using a combination of exposure sequences to 0.24 Gy at 13 days and BrdU injection, he concluded that relative sensitivities were: $G_2 + M > G_1 > S$.

Their results indicated that a number of factors needed to be determined to define and explain radiation effects in the lowest dose range. These include: (1) differential sensitivity of G_1 cells and early postmitotic differentiating cells in the ventricular zone; (2) the existence and radiosensitivity of primitive glial precursor cells in the ventricular zone at the most sensitive stage; (3) the existence and characterization of radiation-triggered apoptosis; and (4) cell loss in the fetal brain and its postnatal functional consequences.

Dr. Kameyama had a somewhat different although not conflicting perspective from that of others, on other routes through which damage could occur at low doses in addition to specific cellular effects. These include alteration of tissue damage repair; cell-to-cell and tissue-to-tissue interactions; and modification of programmed cell death or structure-function relationships. He believed that the role of macromolecules on cell surfaces and in extracellular matrix in neuronal migration, as well as the developmental implications of neurotransmission, must be established relative to radiation effects.

NEUROSCIENCE PERSPECTIVES: MODERN CONCEPTS AND METHODOLOGIES

The second day's presentations were designed to communicate the basic concepts of nervous system development, particularly as determined through more recent molecular and neuroscience approaches. The presentations provided the participants with a broadened and more detailed view of neural development and yielded an appreciation of the experimental techniques that are providing this mechanistic perspective on the normal and deranged sequences. This information was especially useful to those who lacked background because their disciplinary training had been in areas other than biological sciences and to those whose opportunities to follow contemporary advances were limited by demands deriving from their broad concerns with whole-animal responses or emphasis on the toxic agents per se.

BASIC PROCESSES IN NEURONAL ONTOGENY

Dr. Michael Miller of the University of Medicine and Dentistry of New Jersey began this session with an overview of normal prenatal development of the brain that summarized basic ontogenic processes, with emphasis on the individual categories of neurons. The development of cerebral cortex was described as representative of patterns that broadly apply to most brain regions. He also illustrated relationships between the abnormalities resulting from prenatal ethanol exposure and their functional effects as well as a comparison with changes produced by irradiation.

Individual structures of brain begin developing independently, but they soon become integrated with other structures as growth factors begin to circulate. As he illustrated by the microscopic appearance of the telencephalon in the 13-day rodent embryo, the neuroectoderm is a pseudostratified columnar epithelium that gives rise to several histologic types. Terminology has evolved through the years as relationships became more clear, but the structure and nomenclature of both the early embryonic and postnatal brains applicable to his presentation and those that followed are approximated by the generalized diagram, Figure 2.

Dr. Miller described typical experimental approaches to investigation of the processes that determine the subsequent localization of progenitor cells.

Typically, a pulse of tritiated thymidine is injected and chased; autoradiographs are prepared and examined at later times. In confirmation of others' descriptions, the general pattern of neuronal migration is an inside-to-outside process, but exceptions are known. It takes 6 to 10 days to transit from the ventricular zone to the superficial cortex. After thymidine injection at gd 13, the cells are later found in layer VI, or deep cortex near the ventricle. After injections at gd 17, labeled cells are located in the middle cortex, while cells labeled on gd 21 are subsequently found in layer II.

The process that directs migration was originally referred to as contact guidance but is now known to involve radial glial fibers that extend from cell bodies in the subventricular or ventricular zone to the cortical plate, or perhaps even to the pial surface. Rat 401 antibody, among others, is used to identify these transient glial processes or fibers, and the neurons may be seen to be lined up on the fibers like rows of grapes. Molecular probes have shown definite interactions between glia and neurons, which are clonal in nature. The radial fibers are also involved in the movement of the glial cells and their transformation into astrocytes after completion of neuronal migration, at about 10 days of postnatal life.

Labeling studies have identified and characterized two types of neurons. One is the so-called projection neuron, which constitutes about 90% of the population and gives rise to the pyramidal cells. Its axons extend far from the cell body and form asymmetric stimulatory synapses that act through excitatory amino acids such as aspartate and glutamate. As shown by double labeling studies in which tritiated thymidine administration is followed by retrograde injection of horseradish peroxidase, these neurons begin to migrate at about dg 14, while deploying their axons behind them. They move to be positioned in deep cortex and subsequently develop dendrites near the cell body; later generations of neurons, formed at times up to term, are found less deep. By substitution of gold for the golgi, it has been determined that asymmetrical synapses develop together with the dendritic spines, which are the identifying characteristic of projection neurons. There are large increases in spine density in layers II/III and V between postnatal days 6 to 9 and 12 to 15, which coincides with the time of eye opening.

The other type is local circuit, or stellate or bitufted neurons, which constitute 10% of the population. Their formation is also inside to outside during the period from gd 13 through term, which is the same timeframe as projection neurons. However, their axons are located near the cell bodies, and develop later so that they fit into the asymmetric synaptic network formed by the dendritic spines of the projection neurons. Its symmetric dendritic synapses develop later and have an inhibitory action via peptides or gamma-amino butyric acid (GABA), as identified immunohistochemically through the use of GABA antibody probes.

ALZ-50 is an antibody probe derived from Alzheimer brain that does not label anything in the normal adult brain. The probe localizes in the subplate, below the cortical plate, which is the zone of cell death in the prenatal animal. It also stains dying cells, and if the dye is put in dead tissue, it will label widely. Studies with this probe have demonstrated naturally occurring death of neurons subsequent to their migration through the cortex. These cells reach a deterministic point of survival or no survival, which is presumably related to competition for nerve growth factor and to their ability to produce protein. Dr. Miller considers this process to be different from so-called programmed cell death.

Consumption of alcohol by the pregnant woman may disrupt development of the cortex in the human fetus, leading to the fetal alcohol syndrome (FAS); similar effects are produced in alcohol-treated rats. The experimental approach used by Dr. Miller is to fill entire cells with label to study processes in FAS. The architecture looks normal histologically, but labeling identifies spatial distortion; layer V neurons are found in what probably is layer I. Evaluation of changes in structure and function in alcohol-treated animals has shown that these heterotropic cells retain their phenotypic structural character and remain active and functional; however, there is a 28% decrease in glucose utilization. Thus, the cells that have undergone faulty migration after prenatal alcohol insult remain viable, which is in contrast with the postulated changes following irradiation.

CELLULAR AND MOLECULAR FACTORS IN NEURONAL MIGRATION

Dr. Shinji Fushiki of the Kyoto Prefectural University of Medicine in Japan is a pathologist with an interest in the formative processes of brain development. He has been collaborating with Dr. Schull for the past 2 years, under sponsorship by the Atomic Energy Control Board of Canada. He described in vitro and in vivo studies at his laboratory of radiation effects on the cellular and molecular factors involved in the neuronal migration that forms cortical structures. His perspective on normal corticogenesis in the human fetus paralleled that described by Dr. Miller for rodents, but his presentation emphasized the roles of cell-to-cell interactions in histogenesis. In his view, the process involves four components that simultaneously occur in different regions: (1) proliferation, (2) migration, (3) differentiation, and (4) regulation.

Some technical details are varied to suit the question, but their general in vitro assay system for study of neuronal migration uses neocortical (forebrain) cultures. Segments are explanted from 14- to 16-dg rat embryos. They are then labeled with bromouridine or tritiated thymidine, washed, and transferred to multiwell plates. They are subjected to the appropriate experimental procedure, and the cultures are then incubated for 2 to 4 days, fixed in paraformaldehyde, and used to prepare paraffin sections and autoradiographs.

In controls, heavily labeled cells move toward the cortical plate by 2 days of culture. The participation of various cell surface molecules has been identified by use of monoclonal antibody probes, and their distribution patterns display age-specific characteristics. To investigate the functionality of active cell surface molecules, inhibition was achieved by adding the antibody against the neural cell attachment molecule (NCAM), L2, to the cultures. The resulting migration patterns were perturbed relative to controls, and heavily labeled cells were found predominantly in the ventricular zone. After irradiation with 0.05 to 0.20 Gy of ^{60}Co gamma rays, the label is also retained within the ventricular zone, indicating that migration is inhibited. Quantitation of percentage labeling in inner and outer intermediate zones, as well as in the ventricular zone, suggests that 0.1 to 0.2 Gy is the threshold range for this inhibition. Dr. Fushiki and colleagues use a

comparable design for their parallel studies that are performed in vivo, except that pregnant animals are injected with tritiated thymidine and then irradiated. Doses in the 0.15- to 0.20-Gy range result in increased numbers of heavily labeled cells in the ventricular zone and decreased numbers in the cortical plate.

Their experimental studies of cell-to-cell interaction in cortical histogenesis suggest a decreased development of radial glial cell guidance. They are developing a modified in vitro system to investigate the identity of molecules involved in neuronal migration and how they are affected by radiation. These efforts have evolved to the point where the cells migrating on structures, characterized as "cables," can be photographed, and it is possible to identify the radial fibers associated with the columnar organization of neuronal cells by scanning electron microscopy. The system is similar to that described later by others, and may become applicable to identifying and quantifying cell surface proteins or cytoskeleton elements.

Questions were raised and discussed about the nature of the "cables," but they were not resolved. Dr. Fushiki considered that they were neuronal in origin, but others felt they were glial. During discussions concerning the molecules involved in neuronal migration, it was commented that evidence had been obtained concerning two of the surface molecules. One of these proteins, transin, promotes migration; the other, statin, seems to be involved in arresting movement. There are cell attachment molecules (CAMs) other than L2 that play roles in attachment.

Other discussion centered around whether the observed pathologic changes are permanent or whether the system can "catch up." Dr. Fushiki indicated that he had no direct evidence on this matter, but there were comments that the similarity of this dose range to that which produced postnatal alignment and positional defects suggested the consequences might be permanent. This led to other discussions regarding the main radiation targets in the low-dose range, where, in contrast to higher dose effects on the nucleus or gene products, possibilities include changes mediated through the membranes, including translational or transcriptional defects.

MOLECULAR DETERMINANTS OF BRAIN DEVELOPMENT AND FUNCTION

Dr. Roland Ciaranello of the Stanford University School of Medicine is a Board Certified Psychiatrist and a faculty member of that Department, but his research is based on modern biochemical genetics. His topic was the genetic and molecular control of CNS development, with emphasis on deficits and their functional consequences. He noted that severe mental retardation may occur in the absence of lesions at the light microscopic level, and indicated that he would begin where others had left off. Molecular determinants are involved in three important constituent sets of processes of brain development: (1) axon pathway guidance and synaptic specification, (2) maturation of the synapse, and (3) myelination.

The first of these processes, axon guidance and synaptic specification, involves five different sets of molecular signposts that guide axons toward correct targets and to the proper choice among potential targets to form stable or correct synapses. The first set is composed of the several extracellular matrix (ECM) glycoproteins that promote axonal growth, including fibronectin, laminin, and collagen. The second group is composed of the cell surface proteins that interact with ECM proteins to achieve binding. These include molecules such as integrin, which is composed of α and β subunits and yields a wide variety of receptor configurations that confer specificity. The third category is formed of other more general cell surface molecules, which are responsible for nonspecific cell-cell adhesion by promoting homophilic interaction. These include N-cadherin, which are generically referred to as NCAMs or neural cell adhesive molecules. Fourth is the highly axon-specific group of cell surface proteins that go under a variety of identifying letters and names, such as L1, G4, F11, neurofascin, contractin, and TAG-1. Their actions occur at discrete times and/or regions and promote unique axon-axonal, axon-somatic, or axo-dendritic interactions. The fifth category is composed of growth and trophic factors, rather than surface molecules. These generally are small proteins that are present at low concentration in neurons and serve to induce axons to grow toward their source. Examples include nerve growth factor (NGF), which is required for survival and involved in the physiologic function of cholinergic forebrain neurons, and brain-derived neuronal growth factor (BDNGT) which interacts with laminin to support the survival of

dorsal root ganglion cells. Neurons die in the absence of appropriate trophic factors, and these factors generally support axon growth during development as well as, perhaps, during regeneration.

It is clear from the foregoing that these molecular species have diverse but important roles. The processes by which they operate and the details of the gene regulatory mechanisms are incompletely understood. Several clinical disorders have been traced to perturbations of pathway formation. These include genetic mutations that lead to known syndromes involving agenesis of the corpus callosum. The inherited genetic disorder of albinism is characterized by abnormal melanin formation but also involves abnormal crossing of pathways in the optic chiasm. Environmental factors may also lead to conditions such as FAS, which has been described.

The second process, synaptic maturation, arises from the need for axons to form functional synapses with their target; otherwise, they will atrophy and die. Synaptogenesis is a multiple-step, interactive phenomen that provides proper positioning. It is known that membrane function and ion fluxes, which are involved in the action potential, are of importance, and the axon must synthesize and release neurotransmitter. There is evidence that the neurotransmitter may play an additional role in development by directing target cell differentiation and acting as a growth factor. It appears that there is a normal overabundance of synapse formation, and appropriate pruning by rearrangement or loss is required while neurons that fail to form stable synapses are thought to die. Dr. Ciaranello believes that the role of organizing molecules, including growth factors, will become increasingly important for explaining normal development as well as diseases.

There are a number of genetic syndromes that involve disorders of the synapse. These include Tay-Sachs disease, which is characterized by meganeurite formation; dendrites with abnormal spikes are formed and survive while normal dendrites die. Abnormal dendritic structure or function is likely involved in a variety of other lesions ranging from the hereditary idiopathic mental retardation syndrome to the "stagger" mutation in mice. Down's syndrome and tuberculous sclerosis are other abnormalities in which synaptic disorders are involved.

The third process, myelination, is accomplished through specialized cells that are wrapped around the axons and secrete myelin. The primary secreting cells are the oligodendroglia in the CNS and Schwann cells in peripheral nerves. A wide variety of adverse conditions, ranging through hormone deficiency, toxins, metabolic diseases, and autoimmune diseases, as well as the "shiver" mutation of mice, all may have deleterious effects on myelination.

Dr. Ciaranello noted in his introductory remarks that there are several disorders that have severe clinical consequences but involve little morphologic change. The increasing availability of molecular biology techniques is providing strategies and methodologies for investigating the underlying mechanisms. There are three primary approaches being used: (1) linkage and genetic strategies, (2) brain-specific developmentally regulated genes, and (3) regulation of genes.

The first approach, linkage and genetic strategies, currently requires the investigators to develop both clinical and laboratory diagnostic procedures, but it has characteristics that should ensure that it continues developing as a powerful tool. In brief, the difficulties arise from the fact that the gene product often is not known, the entity may be multigenic, and there is a lack of diagnostic specificity in neurology and psychiatry so that it is difficult to classify individuals as affected or unaffected. Investigations in Dr. Ciaranello's laboratory have concentrated on a form of infantile autism in which the same parents have multiple affected offspring, and it tends to appear in a 4:1, male-to-female ratio. The statistical approach to its genetics requires many families and they now have an adequate number, about 100 in the United States, including a cluster of 30 families in Utah in which the parents are also autistic.

The resulting segregation and linkage analyses are then combined with genotyping. The requisite laboratory correlates are developed from the fortuitous occurrence of restriction fragment length polymorphisms (RFLP) at about every 500 bases. Restriction enzymes result in longer or shorter fragments that can yield probes, although there are about 5% error rates in RFLP and southern blots that must be controlled by blind evaluations. Mapping and localization studies have compared individuals identified as carriers and those with the phenotype. In addition, cloning of large fragments can produce

gene products for study of the role of the gene in pathophysiology of diseases such as neurofibromatosis and cystic fibrosis.

The second approach involves brain-specific developmentally regulated genes, ones that are present only during unique stages of development. The characteristic mRNAs are obtained from experimental animals and then used to build a cDNA library. Subtractive hybridization techniques are used to obtain an enrichment that amounts to 70- to 100 fold in practice. This library is then used to probe on and off times, an important but laborious process.

The third approach involves determination of genes that are important in nervous system function. This is in its infancy but is being applied to neurotransmitter receptor genes. This approach, especially when used in combination with the others, is considered to offer possibilities to understand normal developmental functional mechanisms as well as those pertaining in derangements.

Replies to questions concerning the location of the autism gene indicated that studies have largely excluded chromosome 13, but are now directed toward chromosome 22, which is thought to contain salvage pathways. In response to a question of why there is a male predominance of autism, Dr. Ciaranello indicated that autism does not behave as though it were linked to the X chromosome. Other discussion followed concerning the possible functional significance in near-term male rat fetuses of the late testosterone surge that is involved in masculinizing the brain.

MOLECULAR BASIS FOR CELL-SPECIFIC CYTOTOXICITY AND TERATOLOGY

Dr. Phillip Mirkes of the University of Washington's Central Laboratory for Human Embryology had been asked to illustrate how a logical sequence of experimental approaches could be used to investigate biochemical and molecular mechanisms underlying the response of embryonic organs and cells to teratogenic agents. He did this in the context of describing his laboratory's comparisons of the differential responses of the CNS and heart to cyclophosphamide (Cp), an antitumor alkalating agent that cross-links DNA and requires activation by P450. It is a teratogenic agent that produces several

malformations, ranging from exencephaly to cleft palate in rodents, and there is evidence for induction of similar teratisms in the human fetus.

In vitro studies by Dr. Mirkes and colleagues utilize rat embryos that are grown as whole-embryo cultures, which include the intact yolk sac. Following removal and culturing on days 8 to 12, the cultures exhibit development that is indistinguishable from in vivo over short time periods. In the studies he described, the cultures were exposed to Cp on day 10 and evaluated on day 11. The drug produces decreased growth, abnormal morphology, particularly of forebrain, and cell death in most organs with the exception of heart, which seems immune from Cp-induced cytolethality.

Several studies were performed to quantitate changes. The approaches included ^{14}C-thymidine labeling of DNA before Cp administration, followed by autoradiography and counting of radioactivity in the embryonic heart and head, which, because of technical limitations, was used as a surrogate for neuroepithelium. In the hearts, no differences were detectable between the control and exposed cultures at 5 hours and only minimal differences were found at 24 hours post exposure. The heads displayed little difference at 5 hours, consistent with that being the beginning of histologic effect, but there was markedly less activity in the exposed than the controls at 24 hours, consistent with their high incidence of cell death. After administration of tritium-labeled Cp, light microscopic examination and grain counting of autoradiographs of sagittal sections at day 11 demonstrated that Cp reaches the heart. This conclusion was confirmed by measurements of activity per microgram of protein.

It is known that Cp interacts with several other cellular macromolecules in addition to DNA, its primary target. This led the investigators to perform studies in which homogenates of radiolabeled embryos were layered on buoyant density gradients. Most of the radioactivity from whole embryos was associated with protein, although some was detectable in the DNA and RNA bands. The same ratios of activity were found when this was repeated with separate analyses of heads and hearts. These findings required the researchers to consider the possibility that protein could be the target for teratogenesis. Accordingly, heads and hearts were run on two-dimensional gels and then autoradiographed; no quantitative differences were detected.

The next experiments were suggested by several lines of evidence. In brief, an intermediate metabolite of the drug is the active antitumor agent, deactivation or detoxification is accomplished by aldehyde dehydrogenase (ADH), which occurs as several isozymes, and there is a tumor cell subline that is not responsive because of elevated concentrations of ADH. Measurements in hearts, however, were unable to detect elevated levels of enzyme relative to other tissues. Antibody preparations to the enzyme were used to probe histologic sections; these intensively labeled gut epithelium and extraembryonic areas, but did not selectively bind to heart.

It is thought that the selective antitumor action of Cp is caused by effects on rapidly proliferating cell populations. This led to measurements of cell cycle times by percent labeled mitosis techniques, supplemented by flow cytometry and use of chemically synchronized populations. At this stage of gestation, the cell cycle time of 13.5 hours in heart is 4 hours longer than the 9.5 hours in neuroepithelium. Most of the difference is attributable to G_1, but there may be a loss of proliferating cells that subsequently remain in G_0, and there is the possibility that earlier differentiation of heart may play a role.

Ongoing studies are using alkaline elution, with and without preirradiation, to study the involvement of single-strand break and repair, and differential DNA-DNA or DNA-protein cross-linking in the organ responses. The still incomplete data strongly suggest that two-thirds of the cross-linking is DNA-DNA in the head, but only one-third in the heart. There is measurable repair by 24 hours, and its extent is not different in the two structures. The evidence suggests, however, that the apparent repair may occur through removal of dead cells in the brain, while there has been true repair of strand breaks in the heart.

Dr. Mirkes' presentation was followed by an extensive discussion period that concentrated on relative times of maximum organ sensitivities and embryo lethality. A critical point relative to the rodent is that the time of greatest sensitivity for brain function deficits was in the period that followed highest sensitivity for lethality. As a result, the abnormal embryo/fetus may not die and so may be available for detection as abnormal. From results described in the literature, it seems that a similar situation may apply in the

human. This was followed by discussions about whether there are reasons to expect that there is a threshold for functional deficit induction. For the sake of argument, it was suggested that because cortical neurons are clonal in nature, one cell would have many descendants that could lead to a detectable change. Verbal calculations indicated that the percentage might be too small to produce an effect, but the discussion ended without a conclusion being reached.

MECHANISTIC APPROACHES AND CONCEPTS TO INVESTIGATE ABNORMAL CNS DEVELOPMENT

Dr. Anthony Verity, Professor of Neuropathology at UCLA, addressed mechanistic approaches and concepts for studying CNS development and reviewed some of the modern techniques that are used to characterize the pathogenesis of abnormalities. He illustrated these approaches with methyl mercury and discussed the great overlaps of exposure results to those after radiation. In a sense, these considerations all address the question, "Why does the neuron die?"

Many studies of CNS pathogenesis use various forms of cultures; some of these, such as organ, explant, and whole-embryo cultures had been discussed earlier in the day, while reaggregation techniques were not represented in presentations at the workshop. Dr. Verity discussed studies using two other cell systems that he and his colleagues use as surrogates for neurons: dispersed cell cultures and dispersed cell lines; although other cells (glia) are present and important, they were not to be considered per se. In his view, regardless of their source, neuroblasts are undifferentiated neurons. The cells may be obtained as early or primary cultures from neuroblastomas, while stable lines are also available.

Differentiation and biological behavior of these cultures can be manipulated by addition of retinoic acid or vitamin A, which stimulates the formation of cell processes and aggregation, characteristics associated with differentiation. Astrocytes are of glial origin; they are usually present in the cultures and their presence is required for neuronal aggregation into clusters. These aggregates show fasciculation and a "cabling" phenomenon seemingly similar to that in the experiments Dr. Fushiki described, and which is considered to indicate the importance of intercellular interactions.

Many of Dr. Verity's studies use granular cell cultures that result when pieces of cerebellum are excised from 3-day-old animals, dissociated, and plated into DME media with serum. The cultures can survive for up to 28 days, depending on conditions and the presence of chemical factors. When the cultures are first initiated, the cells have a rounded appearance, similar to lymphocytes. Processes develop during the following days, which gives the cells an ameboid appearance, and this is followed by aggregation of cells into clusters with distinct processes or fasciculations.

The progression of the culture depends on several components of the culture medium. Thyroid hormone or T3 is required for aggregation. In its absence there are disintegration of processes, no aggregation, and cell death between 14 and 21 days. Addition of T3 to medium that is deficient in thyroid hormone leads to process formation and aggregation, which results in longer survival of the culture. If methyl mercury is added, however, it is possible for cell death to occur before processes disintegrate, so that the component events can be dissociated.

A depolarizing medium containing K^+ ion is needed for the cells to differentiate and form processes, and there must be a Ca^{2+} gradient to develop aggregates. These effects can be mimicked by NMDA (or glutamate), again implicating the role of neurotransmitter in differentiation, as discussed by Dr. Miller. There are marked similarities between the effects of methyl mercury and radiation on neural migration, architecture of the neural plate, generation of ventricular cells, and effects on aggregation, all of which provide phenomena that may serve as a basis for experimental exploration.

Alkaline elution studies of single-strand breaks (SSB) produced by 0.45 Gy show that substantial repair occurs within a 60-minute period, but repair is blocked by addition of mercury. Mercury alone induces SSB, and sensitivity to methyl mercury is about 30 fold higher. In contrast to radiation, however, there is no repair of mercury damage after its removal. On the other hand, there is no evidence of mutagenesis or carcinogenesis by inorganic or organic mercury.

Dr. Verity feels that another important component of the interactions with the two types of agents is the role of free radicals. Methyl and

inorganic mercury produce significant amounts of lipoperoxidation, which is the end result of free radical generation. In turn, this leads to reduced levels of reduced glutathione (GSH), paralleling radiation mechanisms, and there are indications of reasonable correlations between the time of onset of cytotoxicity and the magnitude of the lipoperoxidation. In addition, a 32-kdalton stress protein is induced by methyl mercury and by H_2O_2. The data thus suggest that the similarity of x-ray and organic mercury effects may relate to a possibility that they operate through a common final pathway involving oxygen radical induction. It has been shown in hepatocytes that antioxidant inhibition of lipoperoxidation, which is considered an indicator of effects involving membrane, is not cytoprotective.

It is possible that lipoperoxidation and membrane damage may be epiphenomena and not directly responsible for the cell killing. This led to reconsideration of the other two major pathways of injury - effects on DNA and protein degradation or accelerated proteolysis, where membrane damage may be the coupling mechanism. It has been shown that UV cytotoxicity, an oxygen-dependent process, involves a cytoprotective effect of endogenous GSH. Also DNA fragmentation or SSB produced by H_2O_2 was blocked by Quin-2, and there is evidence through which this may be interpreted as calcium acting as an intermediate in producing the breaks. In summary, the radical may have a direct effect through a membrane-protein-cytotoxicity sequence, and/or it may act through calcium to cause DNA strand breaks and cytotoxicity. It is known that altered calcium ion concentrations can lead to activation of endogenous proteases, phospholipases, and endonuclease, all of which can result in cell death or apoptosis. Several lines of evidence were presented that strongly implicate the endonuclease as being the proximate toxin for neuronal cell death both with mercurials and with ionizing radiation.

The question period brought up contradictions regarding the roles of NMDA, which range from being protective against the absence of ionic species in early culture to known toxicity in mature cells. Although it was entirely speculative, the discussion that followed raised intriguing possibilities regarding membrane permeability, ion channels, and receptor sites relative to stage of differentiation and to the process itself.

APPLICATION OF NEUROSCIENCE CONCEPTS AND METHODOLOGIES

The final individual speaker session consisted of a single presentation by Dr. Peter Spencer of the Oregon Health Sciences University. He had been requested to draw on his broad experience to synthesize a neurotoxicologist's view of the prenatal radiation problem and suggest how the remaining questions might be addressed through newer concepts and methodologies. An important concept in his thesis was that early effects, including mental retardation, might not be the only consequence of prenatal or early postnatal exposure to noxious agents and that late effects of early exposure may be of particular importance. These late effects may remain silent for years or decades, appearing only later in life as progressive deficit. The presentation provided stimulus for the subsequent discussion periods for developing research strategies and recommendations.

Neuronal aging and injury display definite and characteristic patterns. These are most readily illustrated in peripheral nerves, although they may be of lesser interest. Injury often results in the loss of the myelin sheath, with morphologic changes and conduction block, but it often tends to grow back. With axonopathy, especially of long fibers, there may be sensory loss. Often there is retrograde degeneration, in which the distal portion dies back, although the process may be reversible. In contrast, CNS neuronopathy is more important, and aging has striking effects that result from the progressive loss of neurons after maturity. There is a normal redundancy in number and the subject may compensate for long periods of decrease, but there will be clinical effects when the number decreases sufficiently. As an example, cell loss in the dorsal root ganglion of animals, and perhaps in humans, contributes to the sensory deficits observed in aged subjects.

Poliomyelitis is an example of a disease in which motor nerve cell loss occurs in early life, but various compensatory processes allow partial recovery. There is then a long latent period during which additional neurons drop out and the victim displays a rapid functional decline later in life. Evidence is increasing that the effect of specific age-related lesions is associated with particular major nuclei in humans, resulting in the specificity of the declines with age. Specific nuclei in the brain have been shown to

be associated with Parkinsonism and Alzheimer's disease, while cell loss in a spinal cord nucleus is responsible for amylotropic lateral sclerosis.

It can be speculated that developmental toxins might not yield overt deficits because of the large safety factor or numerical margin. It would be predicted that aging effects could lead to earlier expression in individuals in whom initial numbers had been reduced by the toxic event, which would provide a basis for long latent periods. The polio situation, as well as post-encephalitic Parkinsonism, provides evidence for this situation, and positron emission tomography (PET) scans demonstrate characteristic changes in patients with these diseases and can demonstrate the affected regions. Some drugs of abuse that destroy cells in specific regions can rapidly induce CNS deficits that strongly resemble Parkinsonism, but the scans show somewhat different patterns.

A nervous system syndrome has been recognized in an isolated population in Guam, and to a lesser extent in one of the Japanese islands, and in an area of New Guinea geographically distant from that associated with kuru. This is presented as neuromuscular disease with symptomologies resembling Parkinsonism, dementia, and other age degeneration syndromes. More recently it was found that PET scans of affected individuals also showed these characteristic changes. Investigation in Guam traced the origin to the plant cycad, which is known to have toxic components that lead to a locomotory defect in grazing animals. As a result of occupation during World War II, the population increasingly made use of a partially detoxified portion of the plant as a foodstuff, in addition to external use of other parts for medicinal purposes as a poultice. The active components includes cycasin, which is a known carcinogen, teratogen, and mutagen.

The cycad hypothesis was not fully accepted because the Japanese populations did not use it as food and the plant was only recently found in New Guinea. However, the medicinal use alone, during childhood, was found to give rise to disease during young adulthood in some individuals. The symptoms have a latency of 15 to 20 years, and the incidence of disease is decreasing because of a decreased use of traditional medicines involving cycad. Dr. Spencer illustrated the extensive sequence of parallel laboratory studies performed to establish mechanisms and to characterize the pathogenesis

of the toxicity. These include determination of biochemical changes involved with activation, molecular interactions with receptors on synaptic membranes of target nerve cells, and pathogenesis of lesions in cultured cells. These studies have examined the neuropathophysiological changes that are involved, as well as their histologic and cytologic temporal sequences.

Having determined effect on dividing cells, obvious questions arise. What is the effect of such agents on cells that cannot divide or are in terminal stages of division during early development? Can they modify genomic expression in such cells so that the cells subsequently produce material leading to progressive neuronal degeneration? Methyl adduction has been postulated as one possible route, and such processes may provide the links between chemical toxins and nonchemical agents such as ionizing radiation. This provides the rationale for the interest that Dr. Spencer expressed in his introductory remarks about possible late changes that would result from combined effects of latent injury and age changes in the perinatally exposed Japanese population.

During discussion, it was pointed out that, in two early experiments involving continuous irradiation of 10 generations of mice and 11 generations of rats, detectable effects were not produced at the lowest dose rate of 0.02 Gy/day. Progressively severe effects were produced at higher doses, which ranged up to 0.5 Gy/day. As was noted in presentations, however, there are certain combinations of prenatal stage and dose range that can yield changes that are measurable in adult animals.

SUMMARIES OF DISCUSSIONS

An extended discussion period began on Tuesday afternoon to select topics that would collectively summarize consensus regarding the status of current knowledge, identify unknowns and uncertainties that required resolution, and prioritize questions that should be addressed, especially those requiring investigations using newer concepts and approaches. That evening, the participants met as two smaller groups to discuss the topics and questions that arose during these discussions. During the final sessions on Wednesday morning, summaries of the groups' deliberations were presented and integrated, and some consensus was reached as to their importance or usefulness. The essence of these agreements is presented in the following subsections. The relative importance of the potential contribution of future research activities was discussed, and suggestions were developed for approaches to investigation. The recommendations and approaches have been combined with similar considerations from previous discussion sessions; they are rearranged into the revised categories that follow the summary of current knowledge.

CURRENT STATUS OF KNOWLEDGE

1. STATUS OF CURRENT CLINICAL INFORMATION AND RESEARCH IN PROGRESS

A. Several endpoints have been measured in the prenatally irradiated Japanese populations. These results and those of continuing clinical followups are being subjected to a wide battery of statistical manipulations. These clinical protocols are similar to those being used for evaluating persons who were prenatally exposed to other toxic insults, but perhaps are less sensitive. Investigators continue to compare results with the historical database to examine consistency of pattern and potential interpretations.

B. Mental retardation is the most readily determined indicator of decreased mental capacity. This condition is identified by an IQ below a defined level, and severe mental retardation is the extreme case. Radiation effect on mental retardation has been quantified in terms of its incidence; it is clearly increased in the Japanese populations exposed to high doses, which is consistent with previous clinical literature. Dose-response relationships in the low-dose range are predicted by statistical inference, and their precise nature continues to remain uncertain. The period between 8 and 15 weeks of gestation is the most radiosensitive for producing deleterious mental effects.

C. Mental retardation is accompanied by microcephaly, or small head circumference, in about 50% of the cases, although microcephalic individuals may have normal intelligence. It appears that the presence of the two defects is primarily a general association, both resulting from radiogenic alterations of developmental processes. They do not, however, seem to derive from common or concurrent initial events, or to have the same pathogenesis.

D. Some mentally retarded individuals, as well those with other defects, undergo unprovoked seizures. Results from a few autopsies in Japan and from earlier autopsies, indicate that heterotopic and ectopic neuropathologic lesions are associated with the clinical signs. Anencephaly and hydrocephaly have been found in rare cases, but it has not been established that they relate directly to lesions involving misplaced cell populations.

E. The clinical data have not provided clear evidence of deleterious effects from exposures in the 0.1- to 0.2-Gy range or below. The numbers of prenatally exposed Japanese people in this range are relatively small, and it may be useful to study data from other human populations.

F. The long-term consequences to the human CNS from prenatal irradiation at levels that do not produce overt effects are not known, but animal studies suggest the possibility of premature degeneration. There are indications of premature decline following prenatal human exposure to some other agents, which suggests the possibility of similar effects with radiation. Individuals in the Japanese clinical populations, and those in other populations, are still young enough to be considered in addressing this question.

2. STATUS OF ANIMAL DATA AND RESEARCH IN PROGRESS

A. There is good general agreement of results among animal studies. In most instances, the dose levels at which unequivocal permanent damage could be demonstrated have been in the 0.15- to 0.25-Gy range, and pertained with essentially every method reported, in vivo or in vitro, and using structural or functional endpoints. More recent endpoints, such as quantified measures of neuronal and fiber alignment, yield a similar range for no detectable effect, but initial indications suggest that corpus callosum, which has been studied in lesser detail, might be affected at slightly lower doses.

B. Most studies find that reproducible alterations of the cell cycle also require doses in the 0.2-Gy range. The cell compartment composed of M + G_2 seems to be the most sensitive during corticogenesis. There are suggestions that increased cell death may be demonstrable at a minimum of 0.03 Gy, but acceptance is not unanimous. Arguably, spontaneous cell death incidences in the 3% to 15% range would obscure detection, and the question was raised as to how one could see the effect of 0.03 Gy in vivo? The biological significance of cytotoxicity at low doses or of "minimal" morphologic effects, especially those determined primarily by statistical analyses of overall dose-response curves, was questioned relative to persistent endpoints. The question has both theoretical and practical implications in terms of whether the small potential effects exist, or whether they are present but are not detectable with current methodologies.

C. There was consensus that thymidine-labeled dead (pyknotic) cells were present in the ventricular and subventricular zones of the prenatal cerebral cortex at 3 hours after irradiation and that in vivo exposures led to many labeled pyknotic cells in the intermediate zone and cortical plate at 24 hours. Most considered that migration is an active process, but there was disagreement regarding cell-type identification in the interpretation of autoradiographs of histologic sections during corticogenesis.

It was accepted that there was no clear identification of cells in the migratory zones - neuroblasts, glioblasts, blood vessel cells - which led to unanswered questions about how dead cells got there. Suggested possibilities include: (1) an unidentified "something" moved them; (2) there are populations of cells in the periventricular zones with differing sensitivities, and cells seen at 24 hours are not the same as those noted to have died at earlier times; (3) damage is induced during the mitotic cycle but the cells do not die until migration, or (4) incorrectly placed cells are somehow selected against and die.

D. The site and the identity of cells that die and the time at which they die were of concern because of their importance for understanding mechanisms and drawing conclusions regarding response relationships. The times and mechanisms of death and whether removal of migrating cell populations occurred via phagocytosis or apoptosis were left as open questions. Two other questions were left unanswered: Is there sequential death of "different" neuronal "stem cell" populations? Are processes of cytolethality and their relations to mitosis in the embryo different from those in adults or in cultures?

E. Basic neurobiology and neurotoxicology are being actively investigated in the United States, as is CNS-functional teratogenesis to a lesser degree. Toxicologic approaches are primarily directed toward agents of pharmacological, occupational, or environmental concern, and are tested using traditional neurobehavioral measures, sometimes with incorporation of automation and electronic acquisition. With few notable exceptions, little ongoing research is directed toward radiation effects on brain development.

F. Most current research on CNS effects of prenatal irradiation is conducted in Japan and Europe. Studies of irradiated animals in Canada involve neuropathology and migration. Some research in Japan is typified by Dr. Kameyama's presentation, while Dr. Fushiki's research seems to represent the new wave. Several studies in Europe are sponsored by European Late Effects Project

(EULEP); these include those of Dr. Konermann and others directed toward cytoarchitecture. Much of this research currently attempts better quantitation and establishing etiologic correlates and basic mechanisms of previously identified affected endpoints. Most studies consider exposure stage and include brain weight, reduced cellularity, cytoxicity, laminar structure, and cortical thickness. Other measures involve differentiation or migration disturbances resulting in effects on alignment quotient, dendritic arborization, and ectopic placement in disorganized tissues and structures.

3. AREAS OF SIMILARITY AND DISSIMILARITY BETWEEN FINDINGS IN HUMANS AND ANIMALS

A. When appropriate scaling of developmental stages is used as the basis for comparisons, there is a remarkable similarity between the development of human and rodent brain, as well as that of other mammalian species. Some categories of functional deficits, such as altered behavior and seizures, are similar and perhaps identical throughout. There are substantive questions about relationships between cognitive and reflex measures; they emphasize our inability to measure cognitive functions in experimental animals.

B. There is good quantitative consistency between radiation effects in humans and in animals. The threshold range across species seems to be about 0.15 to 0.25 Gy. There are differences between the nature of mental retardation in human as compared to functional or behavioral changes measured in animal species. Many findings show there are close qualitative similarities of neurobiological changes in humans and rodents, and the few available studies have detected close quantitative relationships in human and nonhuman primates, although this may be relevant only for acute exposures.

C. Discussions concerning dose-response relationships were stimulated by comparisons to methyl mercury and the precedents evolving from evaluations of Minamata disease. Prenatal exposure is accepted as yielding a dose-response relationship that is characterized as hockey-stick shape. This term was selected to avoid need for deciding the question of threshold as it allows for future adjustments when mechanistic knowledge increases. Such a hockey-stick response also applies to a great number of other developmentally toxic agents in both humans and experimental animals, although linear, nonthreshold shapes can be justified on a statistical basis and may be useful for some purposes, particularly estimations of risk.

D. It was concluded that the cerebral rosettes observed in experimental studies after irradiation during organogenesis disappear by term and differ from the heterotopia observed postnatally in irradiated humans. Studies involving experimental exposures in later gestation have detected clumping and ectopia, together with cerebellar signs in animals allowed to develop postnatally, including unprovoked and audiogenic seizures. Some speculated that seizures and ectopia/heterotopia may be related, and that stage differences in response are demonstrations of the progressive loss of repair capacities.

4. MECHANISMS TO EXPLAIN QUANTITATIVE RELATIONSHIPS OF RADIATION EFFECTS ON PRENATAL CNS

A. Evidence in animals and humans suggests a possibility of primary neural cytotoxicity, but precise dose-effect relationships for individual neuronal type are uncertain. The nature and magnitude of effects seem to differ relative to stage of cell cycle at irradiation, the relationship to time of migration, and whether the cells are premitotic or postmitotic. An adequate dose to a mitotically active cell leads to DNA degradation and lethality.

B. In all species studied, it appears that secondary effects on the neuroblast may be mediated through nonlethal DNA/RNA modification via indirect actions that alter membrane and/or protein. There is a need to understand direct or indirect effects relative to free radical production and other potential mechanisms of radiation effect.

C. In the developing brain, there are opportunities for induction of secondary effects by nontraditional mechanisms. These are thought to include neuronal events that depend on the integrity of other cells and cell populations, such as guidance of migration and localization, as well as the importance of proper synaptogenesis in survival of neurons. There are no reasons to expect differences among species.

D. The changing ability for repair and reconstitution of radiation-induced damage during development is known to have a general effect on the chronology of cytologic and histologic characteristics and the ultimate functional consequences. The role of the processes involved in modification of injury, as well as modification itself, has not been completely defined and requires quantification.

5. POTENTIAL OF ANIMAL EXPERIMENTS TO IMPROVE INTERPRETATIONS AND CORRELATIONS

Discussions directed toward developing additional information to allow deriving maximum benefit from studies of human populations focused on the applicability of other species and on experimental design considerations. Participants agreed that data on altered behavioral endpoints in animals were not directly applicable to addressing questions associated with mental retardation, despite similarities of morphologic and biochemical effects. The critical question was: Can animals be mentally retarded? It was accepted that there were inherent difficulties in equating animal and human tests of intelligence at our current level of understanding of brain/behavior relationships.

It was agreed that it would be attractive if we could establish a way to examine higher cognitive processes, memory, or complicated tasks as a way to bridge the gap between mental retardation and results of animal studies.

SOCIETAL AND REGULATORY CONSIDERATIONS

1. THERE IS A NEED FOR IMPROVED RISK ESTIMATES

There was agreement that there was need for improved risk estimates for a wide range of postnatal effects produced by intrauterine radiation exposures, exemplified here by the function of the nervous system. From the regulatory standpoint, accurate and realistic risk estimates are important for setting limits that will protect the relevant segment of the population but still avoid the economic cost of unduly restrictive values. The questions regarding dose and mental retardation are a critical element in establishing procedures for radiation protection, as well as acceptance by society.

There were strong feelings that knowledge of threshold and risk also involves broader aspects of societal concern. If a dose of 0.05 to 0.1 Gy produced no changes, exposure of 1,000,000 women to 0.01 Gy would have no effect on population intelligence. On the other hand, if there were no threshold, the argument could be made that pregnant women should not be exposed to even low doses, such as those received during travel in aircraft. The needs and outlook of clinicians, as well as their interactions with society, patients, or other physicians, are also involved. It was noted that it is difficult to take a stand on the absence of effect when speaking with a woman who asks about whether her fetus could be normal after a radiation exposure, and responses must involve asking what is the risk of not doing the procedure or how much is one willing to do to reduce the risk. Thus, there are similar needs for accurate information in advising pregnant women who may be exposed in their environment, occupation, or during medical procedures.

2. TYPES OF INFORMATION NEEDED FOR IMPROVED RISK ESTIMATES

The information needed to improve risk estimates includes a better definition of the nature of the limiting effects, whether there is a threshold, and more precise knowledge of pertinent dose-response relationships. An important aspect toward utilization of estimates is knowing what levels of risk would be considered as acceptable by society. This currently requires an improved ability to extrapolate consequences from high to low doses and from acute to chronic exposures.

The numbers of Japanese survivors are small, and many participants felt that it would be worthwhile to evaluate other prenatally exposed human populations to obtain comparable information. This limitation suggests the desirability of ancillary animal studies to identify sensitive endpoints that would help in guiding human investigations.

3. MAXIMIZE INFORMATION OBTAINABLE FROM JAPANESE SURVIVORS

It was agreed that all possible information should be derived from studies of the Japanese survivors. The individuals are now middle-aged adults, and it is hoped that a population of this nature never again becomes available for evaluation. The data may be of unequivocal relevance only to these restricted groups and exposure conditions, which may be unique and perturbed. The Nagasaki and Hiroshima populations are identified study groups that are amenable to repeated measures to provide data with lasting implications. Primary needs are to define how best to obtain the greatest amount of information, what and how to obtain and preserve specimens for subsequent evaluation by new analytical methodologies, how best to provide additional contemporary data, and what data to obtain. It was suggested that multiple regression analyses should be included to examine interactions among the numerous factors and parameters that had been identified, and that exploring of reasons for differences in results between the two cities might yield meaningful information.

4. EVALUATION OF OTHER AVAILABLE POPULATIONS

It was felt that many issues posed by the Japanese data could be answered with other populations, and that experimental findings might suggest additional measurements in people. The usual situation is, however, that studies of people have identified the phenomena that were subsequently studied in animals. There was incomplete agreement about the value of evaluating other prenatally exposed populations that have been identified because of group sizes. The two groups that Dr. Brent had mentioned - the University of Chicago and the NIH Collaborative Perinatal Project populations - were considered as the most likely for providing useful results in the foreseeable future.

Participants considered the possibilities of obtaining ancillary clinical information from situations involving neonatal exposures. It was recognized that other factors could have caused any effects, and the situation may be unclear at high doses. The most meaningful populations may be those who received ionizing radiation to treat hemangioma of head and neck. It was thought that ongoing studies in Sweden relative to cancer involves large numbers of subjects: 5,000 to 10,000, and examinations might have included or could be used for neurologic evaluations. A Russian study by Tereshenko, available only in translation, included exposures to doses beyond 5 Gy. It was asserted that 50% of those in the 0.4- to 1.3-Gy range have undefined defects of neural function. Although tinea capitis is usually found in older children, it was thought that the populations might include some infants who were irradiated at a lower dose range soon after birth.

It was noted that the Marshallese populations, which include children and pregnant women who received internal and surface radionuclide deposition, have been monitored since 1954. There have been clear thyroid changes at higher radiation dose levels, and the project reports might be examined for results of CNS functional evaluations.

There was a lack of agreement, but several participants thought that information could be obtained from the Chernobyl population, despite obvious limitations. Determining dose is a problem because of restrictions on information that is made available. Estimation of dose from radionuclide ingestion is another problem; this is being examined and its usefulness will not be known until later, although similar problems pertained in Japan. It was speculated that over 500,000 persons received more than 25 rad from Chernobyl.

5. SPECIAL SITUATIONS THAT WARRANT EVALUATION

a. Developmental Times or Stages with Special CNS Sensitivity

Some felt that broader gestational windows of exposure to radiation, or the entire period of CNS development, should be evaluated. Most studies have focused on the period of maximum cerebral cortical formation; less attention has been paid to other areas of CNS other than the hippocampus and cerebellum, which have been studied in detail. It was noted that development should be

considered more as a continuum than as a series of windows, and that the "critical period" widened as doses increased. Some participants felt that much might be learned by studies of fractionated doses and of other neuropathologic-function relations.

b. Longer Term Consequences to the CNS Associated with Prenatal Exposure

Long-term functional consequences of prenatal irradiation are of concern, including neurologic damage that becomes more marked during aging. As was noted, prenatally irradiated rats display an earlier senescence, and it is known that critical parts of the human CNS lose cells with time, and that this may be accelerated by exposure to toxic agents. These considerations can be addressed through emphasis on neurologic disorders and deficits during longitudinal evaluations of Japanese survivors exposed in the prenatal and early postnatal periods. Such measures of premature senescence should be incorporated in evaluations of other human populations.

c. Interactions with Other Insults of Practical Consequence

One participant commented that radiation had been shown to increase sensitivity to morphine, and lesser doses of the drug could produce the same degree of impairment, which led to discussions that identified a variety of interactions that had a potential to affect measured responses. Fetal alcohol exposures and decreased gestational nutrition fall into a general hypothetical category involving a series of events that could result in increased endpoint sensitivity. Agents that exacerbate the injury sequence or interfere with repair or restoration of effects of injury may be expected to increase the magnitude of a second insult or reduce the dose required to produce criterion effect.

Some in vitro studies have shown that prior low-dose irradiation has a protective effect against a second exposure. It was agreed that the compensation or overcompensation after irradiation, which may lead to transient hypercellularity, should not be considered a "positive" effect. Low-dose irradiation of the embryo before organogenesis has been shown to increase its susceptibility to the teratogenic action of irradiation during organogenesis. This may have implications for chronic or repeated radiation exposures.

RECOMMENDED AREAS FOR FURTHER RESEARCH AND POTENTIAL APPROACHES

1. CONTINUE STUDIES OF LIFETIME EFFECTS IN PRENATALLY EXPOSED JAPANESE SURVIVORS

There was unanimous support for the concept that studies to obtain maximum information about lifetime effects should continue in the prenatally exposed Japanese survivors. These should include definition of disorders and deficits and the correlations among them, continued surveillance and implementation of assays to detect delayed effects, and ancillary attempts to evaluate the role of interactions with other insults through closer ties between the experimentalist and epidemiologist that would lead to carefully designed experimental studies.

2. EVALUATE OTHER HUMAN POPULATIONS, INCLUDING THOSE WITH EARLY POSTNATAL EXPOSURES

There was general support for suggestions of similar investigations of early postnatal and lifetime CNS effect in other exposed human populations. These include those that received exposures in the early postnatal period to external radiation, or via radionuclides with its unique and phenomenological aspects. Most participants were in agreement, although a few felt that population sizes might be inadequate to provide statistically meaningful group sizes.

Design Considerations for Clinical and Epidemiologic Studies

The RERF currently evaluates 400 to 600 members of the clinical sample on a 2-year cycle. It was suggested that neurologic examinations be recorded on videotape for consultation with other neurologists and for archival purposes to allow review should additional analytical procedures become available at a later date. This recommendation pertained to evaluation of any other populations that might be undertaken, such as those at Chernobyl. Feelings were strong that such examinations should begin at the earliest possible age, which is now adulthood in Japan. No measurements have been done on this population, as yet, to examine aging effects. Based on effects with other agents,

however, neurologic evaluations later in life should be performed to detect acquired or premature disorders or deficits.

Endpoints should include basic movements, behavioral assays, and various evoked potentials. This could consist of a wide range of noninvasive electronic parameters to be selected from among EEGs, ECGs, and electromyograms, as well as nerve conductance measures using magnetic stimulation. Sensory monitoring would be important and should include visual fields, response to photostimulation, and odor identification and Penn smell tests. This may provide for determinations of earlier changes in functions that commonly begin to decline during middle age, such as smell and vision. Measurements that would allow determination of shifts in curves of graded responses were thought to possibly aid in detecting subtle changes. It was also suggested that multiple regression analyses should be applied to past and future data and that more comprehensive statistical comparisons of responses in Hiroshima and Nagasaki, as well as analyses after segregation by potential confounding insults, such as maternal symptomology, might yield quantitative indications of the factors that influence response.

There was corroboration of ongoing and planned efforts to collect and store accessible body tissues such as blood cells and serum, sperm, and hair, as well as surgical specimens. It was noted that tissue registries exist for handling excised tumors. There was encouragement for ongoing efforts relative to the F_1 cohort in which blood is obtained from the father, mother, and offspring with the goal of obtaining samples from more than 1000 trios (3000 individuals) and to immortalize white cells for later study of DNA, perhaps body fluid for neuron-specific enolase, and to provide an archive for analyses that may become available in the future.

Efforts toward obtaining magnetic resonance images should be continued and expanded if possible. Correlations between changes in these images with clinically detectable neurologic changes, and the association of these factors with seizures, will be of particular interest and potential importance. It is recognized that performing autopsies may have ethical, legal, and political implications that require consideration. However, substantial knowledge may be obtained by correlating this information with the lifetime neurologic status and corresponding MRI patterns. The RERF was encouraged to undertake

diligent attempts to obtain permission for performing autopsies on individuals who had any degree of mental impairment, as well as proper controls, while balancing these efforts against proper concern for cultural sensitivities.

3. ESTABLISH INTERACTIONS OF INSULTS IN EXPERIMENTAL ANIMALS

There was strong general agreement that there was a continuing need for studies in experimental animals to investigate interactions between prenatal irradiation and other insults. The results would allow establishing a database that could be used for extrapolations to establish risk. There was strong support that these should emphasize nonhuman primates, so that the resulting data might provide linkage between results from studies of human populations and experimental animals.

Theoretical Basis for Correlating Effects with Mechanisms

It seems unlikely that a theoretically sound basis for establishing all relationships developmental stage, dose rate and dose, and resulting effects can be established. The most common sentiments seemed to be that (1) individual problems would require separation into components for observational or experimental determination, (2) an understanding of the underlying mechanisms would be required for valid interpretations, (3) collaborations would be an important facet of the process, (4) efforts should be made to overcome differences that were primarily semantic in nature, and (5) temporary operational agreements should be made to circumvent problems arising from unknown theoretical bases.

Epidemiologic studies have not unequivocally established any deleterious nervous system effects in humans at doses below the 0.02-Gy range other than by extrapolation of response curves. It was noted that many epidemiologists are unaware of results reported in the toxicology literature and that this information might facilitate interpretation. These considerations might be taken as suggesting a need for studies in animals or other systems to obtain correlative data or results at lower doses, but effects might not be determinable and would require questionable extrapolations. Nevertheless, routes for heightening communication between experimentalists and epidemiologists were considered as desirable for meaningful interpretation.

Moreover, it seems appropriate that laboratory animals be used for studies that would determine interactions between other insults and prenatal radiation, the role of dosing regimens, and other phenomena such as altered susceptibility to subsequent postnatal insults in surviving populations. Disagreements evolving from concepts such as mental retardation, as contrasted to demonstrable or theoretical changes in intelligence, are largely derived from semantic differences and can be readily circumvented. As it is currently defined in the radiation protection community, knowledge of whether an effect is stochastic (probabilistic) or nonstochastic (deterministic) has important implications in determining threshold and curve shape in the low-dose range and in the setting of standards. It is clear that such determinations, as well as those suggested above as possibly being indeterminate, would require a coordinated series of mechanistic studies using the techniques of modern biology for their resolution.

Design of Animal Studies for Quantitation or Mechanistic Evaluation

The similarities between the likelihood of producing morphologic lesions in experimental rodents and humans are clear. The relationships relative to functional endpoints are more unsure, however, so there is a need for studies of other species. These have not been explored in detail, and, in any event, there is a need to match stages of development for evaluations. Essentially all suggestions regarding species of animals that might be used centered around nonhuman primates, for which there are corresponding batteries of tests and measures that might be employed.

The crab-eating macaque was suggested as a likely surrogate species that is phylogenetically closer to man. Its motor and sensory systems are similar, and it may provide for a transition between lower animals to humans. This would allow correlation among structure, function, and behavior. Most primate species would allow use of longer times between insult and measurement or evaluation. Thus, delayed effects might be modeled with nonhuman primates, including their long-term brain function and correlates with morphology. The obvious limitation is that it may not be possible to interpret results from the dose range that produces effects to those extrapolated to the low doses needed for comparisons with humans.

4. OBTAIN INFORMATION TO ESTABLISH DOSE-RESPONSE RELATIONSHIPS AND EXTRAPOLATIONS

There was general agreement that there was a need for studies to provide a basis for using current and future information to establish more meaningful dose-response relationships. Mechanistic studies, such as those that will be presented in the following recommendation, were considered to provide the first steps toward a theoretical basis from which to establish curve shapes and extrapolate from empirical data for animals to women. As was indicated, the pragmatic need is to set limits appropriate for chronic exposures at low doses and dose rates, but acceptable methodologies have not been defined. Investigations that will provide a parallel experimental base are needed to allow extrapolating from acute animal exposure data to predictions for chronic exposures as well as from high-dose to low-dose exposures.

No acceptable approaches, other than those deriving from inferences of mechanistic studies, have been offered, and definitive experimental or theoretical approaches are important needs. Despite this situation, it was agreed that the influence of other insults, including nutritional deprivation, could be examined in such animal studies.

5. INTEGRATED MECHANISTIC STUDIES WITH MOLECULAR BIOLOGY AND NEUROTOXICOLOGY APPROACHES

The participants unanimously and enthusiastically supported the concept that concerted mechanistic studies should be undertaken. It was agreed that incorporation of molecular neurobiology concepts and techniques into collaborative investigations with other disciplines could provide opportunities for understanding processes and defining an integration of mechanisms and effect. It was felt that this knowledge might provide understanding that would facilitate making decisions concerning the shape of dose-response relationships and the possibility of thresholds and their location. Constraints of time at the conclusion of the workshop precluded developing this element with great specificity, but participants offered the generalities presented below, and other possibilities were included in presentations concerning neurotoxicologic approaches.

Molecular Neurotoxicology for Integrating Mechanisms and Effects

As indicated, the participants felt that the concepts and approaches used in modern neuroscience, which incorporate the techniques of molecular biology, had a strong potential for making substantive contributions to our understanding. There was strong sentiment that collaborations could be particularly profitable, that these should be comprehensive efforts in which the research of the molecular biologists was integrated with that of colleagues in related fields so that the findings and overall information obtained contributed to enhanced understanding. It was agreed that a wide range of phenomena could be investigated in this manner. Suggested areas included determination of reasons for changes in functions that ranged from cell division and defects of migration to the associated cell-to-cell interactions. Study is required to test generalizations that had been proposed regarding mechanisms; these included the conclusion that the terminal part of the cell cycle seemed most sensitive, the hypothesis that there is a single common pathway of damage, and that gene expression seemed to be a more important mechanism than cell death. Additional approaches that might enhance results would be to incorporate study of molecular lesions in transgenic animals and the effect of these lesions on the response of neurons to ionizing radiation and alterations in gene expression.

Molecular neurobiology offers possible opportunities for expanding our understanding of recognized processes that occur during the genesis of radiogenic CNS developmental deficits: (1) migration, (2) cell-to-cell interactions, (3) gene expression, (4) cell division, and (5) cell death. To understand such phenomena in terms of molecular biological changes, it is necessary to understand alterations of gene expression in the response of neural cells to ionizing radiation. Prior to their terminal division, this might be examined in terms of secretion of surface proteins, ECM composition, adhesion, and their roles in achieving normal cytoarchitecture. For postmitotic cells, it is necessary to identify other relevant responses. The functions of glial cells, which can also proliferate, must be considered in the overall phenomenon.

A pharmacological approach, using radiation in combination with protective or sensitizing agents to modify terminal response, might possibly provide

useful information. The approach might address weak areas, which include cytopathologic lesions that seem to be dose dependent, and reasons why expression of pathogenesis seems to differ among neuronal group. One approach could be to introduce chemical perturbations for selectively testing mechanistic hypotheses using molecular neurobiology approaches. Resolution of such questions might provide information to facilitate extrapolating from rodents to other species to primates. This route of investigation might lead to means for addressing remaining questions: to what extent are effects cumulative? What are the differences and similarities between continuous and discontinuous exposures? What about acute versus fractionated doses? These are important considerations relative to risk estimates and assessments.

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ACKNOWLEDGMENTS

I am grateful to the U.S. Department of Energy for encouraging and supporting efforts to organize and conduct this workshop. Special appreciation is extended to Dr. David A. Smith, Director of the DOE's Health Effects Research Division, Office of Health and Environmental Research and to Dr. Robert G. Thomas, who was the cognizant Project Officer during the planning and implementation phases.

Dr. Robert Miller began his involvement with these questions in postwar Japan and has devoted continuing professional efforts toward their resolution; he was to have presented the introductory overview. Dr. Carmine Clemente performed some of the early experimental studies in rodents and nonhuman primates and was to present a review of morphologic studies in irradiated animals. Circumstances made it necessary for them to withdraw from participation, but we acknowledge their valuable contributions to the planning.

The other investigators who contributed to our knowledge throughout the past decades are too numerous to list without embarrassing omissions. It is appropriate to acknowledge the few individuals whose contributions were mentioned repeatedly during presentations, but who are not otherwise noted because of report format. These include Drs. Ken Brizzee, Anatole Dekaban, Ernie Furchgott, Sam Hicks, Pasco Rakic, and Roberts Rugh.

Aspects of a common thread of collaborations and mentoring were mentioned during presentations and discussions but are not included in the text. The guidance of Dr. Josef Warkany, which led to the clinical observations in Japan, was noted, but he was also involved in stimulating the research of Dr. James Wilson and the generations of graduate students whom he trained. Among the earliest of these was Dr. Brent, who, with his students and collaborators has greatly contributed to knowledge of the effects of prenatal irradiation. Reports of the findings that Dr. Brent was obtaining on the effects of x rays in his graduate studies with Dr. Wilson were responsible for my undertaking parallel studies of the dosimetry and effects of radiophosphorus under the mentorship of Dr. Thomas Noonan. We serendipitously noted neurologic changes in subsequent studies of the life-span effects of prenatal x-irradiation, and Dr. John Sterling Meyer guided me through their

interpretation. These efforts led to a series of collaborative studies with Drs. Jack Werboff and Joan Havlena on the behavioral effects of prenatal irradiation.

As a closing acknowledgment, I am pleased to express gratitude to my friends and colleagues, Drs. Dennis Mahlum and James Park for their help, encouragement, and support throughout this effort.

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