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# The Effect of Maternal Hypoxia on Metanephric Development in the Hamster

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BOSTON UNIVERSITY  
GRADUATE SCHOOL

Dissertation

THE EFFECT OF MATERNAL HYPOXIA  
ON METANEPHRIC DEVELOPMENT IN THE HAMSTER

by

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Submitted in partial fulfillment of the requirements  
for the degree of Doctor of Philosophy 1960

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### Acknowledgements

I wish to express my appreciation for the support, suggestions, and criticisms of my major professor, Dr. Donald I. Patt, and for the cooperation of others who have made it possible to complete the research program reported in this dissertation. Dr. George P. Fulton, chairman of the Department of Biology, and second reader of this paper, has contributed significantly to the accomplishment of the work through his thoughtfulness, encouragement, and criticism.

I wish to express my gratitude to the reference librarian, Miss Irene Christopher, and the staff of the Chenery Library for their courteous assistance and for those whose wisdom and efforts have been instrumental in establishing the excellent library facilities available throughout the Boston area.

Particular thanks are extended to Mr. Frederick W. Maynard under whose direction and assistance the illustrative material of this thesis has been prepared.

Finally, I acknowledge with greatest warmth the love, encouragement, and sacrifices made by my wife and children during the period of research, experimentation, and final writing of this dissertation.

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## INTRODUCTION

### Statement of The Problem

The factors involved in the unfolding of a complex organism from the fertilized egg are remarkable not only in the predominantly successful outcome of their activities but also in their adherence to the party line, be it hamster, hawk, or hawthorn. The efforts of the experimental embryologists of the late nineteenth and early twentieth centuries to learn more of the distribution of responsibilities or capabilities within blastomeres, blastulae, and gastrulae, by using pins and hair-loops, have inspired their successors to continue the search for greater understanding of developmental mechanisms by using techniques which range from equally simple manipulations to the more complex processes of tissue culture, chromatographic analysis, and immunological relationships.

The adaptability and capabilities of an organism are realized only as the environment within which it exists is varied. Success is awarded to the experimental embryologist in proportion to his ability to ask the appropriate question and to observe for the evidence which will direct him to an answer. The teratologist does not wait for the unusual to happen within the confines of the normal ecological niche of the embryo but rather forces the embryo to come to terms in an environment which the investigator endeavors to regulate.

The remarkable coincidence of genetic variation occurring in an accommodating rather than a hostile environment has been responsible for the successes of what would otherwise have been the most ludicrous experiments

by nature. It is no doubt due to this happy circumstance that the metanephric kidney developed. The phylogenetic and ontogenetic implications of this organ have led to the assignment of "value" to a vestigial organ (the pronephros) and to further understanding of inductor relationships.

The teratogenic tool of maternal hypoxia is used in this work as the environmental variable to challenge the normal regulatory powers of the embryo. The challenge used has been selected so that normal functional relationships of the developing excretory system would be interrupted so the demands of normal morphogenesis for this system might be more fully understood.

Most of the published research on the relationships of pronephros, mesonephros, and metanephros has been accomplished by use of non-mammalian material. This has been necessitated by the relative inaccessibility of the mammalian embryo. A considerable portion of the material available on the embryological development of the mammal has been limited to likely assumptions or the probability that because of similarities in morphogenesis, there are also basic similarities in the processes of induction between more advanced vertebrate classes. There is very little reason to expect that the mammalian embryo follows a different set of rules, but the scientist has long since learned not to generalize.

Hypoxia is a relatively convenient teratogen. The equipment necessary to produce a relative oxygen deficiency by decreasing pressure below one atmosphere need consist only of a jar with a tight fitting lid, an inlet with a valve, a manometer, an outlet, and a filter pump attached to a water faucet. The equipment used in the present work to produce hypoxia

in the pregnant hamsters was rendered only slightly more elaborate by incorporating an electric vacuum pump.

An experiment which is designed to use hypoxia to learn more about mammalian embryology cannot be a well controlled experiment. Among the variables one must consider the capacity of the mother to withstand the hypoxic episode, the reflection of the maternal physiological condition on the fortune of the embryo, the status of the individual placenta, variations in the time of fertilization of each embryo, and the role of partial vacuum versus reduced oxygen tension in the final results.

Nevertheless from this melange of variables it has been possible to identify patterns even though specific assignment of responsibility cannot be made. By virtue of the large number of variables there can be no doubt that a broad range of results has been obtained. The number of questions which are unanswered may be far more thought provoking than the single answer from the most carefully controlled experiment.

The hamster is an ideal animal for this type of experiment. The estrous cycle has a regular four day pattern which continues uninterrupted throughout the year. The gestation period of sixteen days terminates with an average of ten to a litter. The animals breed within seven to eight weeks after birth. The maternal organisms show a high resistance to the hypoxic insult while the embryos are susceptible to the teratogen. In over 100 altitude chamber runs there was only one maternal death.

The kidney was chosen as the target organ because of its interesting phylogenetic and ontogenetic development. The pronephros is described as vestigial in the mammal and is assumed to have a "value" comparable to that seen in the bird. The mesonephros of the hamster is not a functional

kidney so again an assumption is made that it nevertheless has a "value". The inductor relationships of the mesonephric duct by way of the ureteric bud have been well established both in vivo and in vitro by study of mutants by geneticists, congenital defects by pathologists and experimental teratologists, and also in tissue culture. The experimental embryologist has found that each new combination of method and material may yield further substantiation to present theories if not new material which can be woven into the fabric every inquiring mind is seeking to make whole.

It is against this background that the following investigation has been made.

#### Historical Background

The development of embryology as an area of inquiry undoubtedly began with primitive observations of changes in normal cyclical bleeding in the gravid human female and extended to wonderment at similarities or differences between offspring and parents. But even more thought-provoking than these would be the observation of terata, i.e. congenitally abnormal individuals. The deep impression made on the minds of persons who had occasion to see such malformed creatures is evident in the many old-wives tales, some of which are even today divorced with some difficulty from the minds of intelligent individuals.

Development of embryology as a science, however, did not begin until the time of the ancient Greek philosophers. Concern with reproductive efforts, the apparent development of an individual from little or nothing, were both instrumental in the orderly examination of chick

development by persons even before Aristotle. Aristotle (384-322 B.C.), however, is generally recognized as the focal point for the beginning of biology as a science because he began to systematize and classify some of the material which came under his scrutiny. The great philosopher proposed a chart relating living organisms on the basis of their embryological characteristics. He studied the development of the chick embryo. He concerned himself with the relative roles of male and female in the reproductive process. His observations indicated to him that the individual develops gradually from the semen, contributed by the male, "which supplies the 'form' to the embryo" while "whatever the female produces supplies the matter fit for shaping" (Needham, 1959). The cessation of menstrual flow was thought to indicate that perhaps the menstruum was utilized in development of the embryo in the human. Aristotle found no evidence of formed substance within the semen which would suggest participation of any unit of the substance in the development of the embryo, in spite of his reference to the male seed. Aristotle's contributions to embryology were not entirely on the positive side, however, as is realized on examination of Needham's balance sheet where it is held that the philosopher's belittling of the female contribution at fertilization and his conception of a universe based on pure reason (which principle he extended to his inquiries on subjects biological) had retarding effects on subsequent development of embryology, largely because of the enormous regard with which Aristotle was held by later scholars. From Aristotle's time in the fourth century B.C. through the Dark Ages, there was little deviation from the pattern of thought followed by the master.

Historians generally refer to the Renaissance as the period of acceleration in the area of biological thought as well as in other areas. Leonardo da Vinci (1452-1519), whose notes on anatomy reflect the work of a carefully appraising mind, is given credit by Needham (1959) for being the first to make quantitative observations on embryonic growth. Oppenheimer (1955) credits Fabricus (1745-1808) with the first published illustrations on chick embryology, but the author also observes that the reading of structures not yet extant into early stages of development may have performed a disservice to embryology by the encouragement of preformist ideas.

William Harvey (1578-1657) was in a position to give strong support to the concept of epigenesis and has been considered by historians to promote this point of view. Harvey's observation of "Ex ovo omnia" might indicate new status for the female in reproduction; however, the "ovum" which he observed was not the female germ cell but the blastocyst. Harvey associated coitus with reproduction and believed that semen played a role, but his dissections accomplished immediately after coitus never revealed the presence of semen in the uterus or tubes. He was even unable to insuflate the uterus so he concluded that the ovum arose de novo.

The observation of animalcules by van Leeuwenhoek (1632-1723) was not accepted immediately as a demonstration of the male germ cell. Van Leeuwenhoek was not to be dissuaded, however, and satisfied himself that these structures were not peculiar to man but existed in many animals.

The identification of the mammalian ovum, in spite of its large size when compared to the sperm, was not to occur for some time yet.

Meyer (1939) identifies Nicolaus Steno (1638-1686) as the first to demonstrate follicles in the ovaries (of the dogfish) and to affirm "that the 'testis' of women ought to be regarded as exactly the same organ as the 'ovary' or 'roe' of Ovipara." In 1672 de Graaf and Swammerdam described the presence of follicles in the human ovary but both considered the follicles to be the ova. The search for the female complement to the animalcules was not based on contemporary discoveries alone, for even Galen (130-200 A.D.) considered the testes and ovaries to be of significance and "pointed to their role in the body as indicated by their loss through castration" (Meyer). Galen also could not conceive of similarities in appearance of offspring to the mother without some contribution by the maternal organism equivalent to that of the male. It was not until 1837, however, that Karl Ernst von Baer identified the mammalian ovum and even then the significance of the discovery was not appreciated.

The problems involved in resolution of disputes between epigenesis and preformation are likewise fascinating aspects of the history of embryology. It seems appropriate at this point, however, to pass on to the truly inspirational ground work for modern experimental embryology which was established by Roux, and carried forth by Spemann, Dreisch, Vogt, and others during the late nineteenth and on into the twentieth century.

Roux's experimentation with the amphibian egg in the late 19th century was directed to ascertain the responsibilities and capabilities of the blastomeres as cleavage progressed. The results of Roux's pricking experiment revealed that "a circumscribed part of the embryo at a definite moment of development contains all specific conditions for further

differentiation" (Spemann, 1938). Driesch performed isolation experiments with the blastomeres of sea urchins and found that complete individuals would always be formed from early blastomeres. It was subsequently found that the amphibian blastomeres under certain circumstances could be made to perform similarly to those of the sea urchin if the injured or dead blastomere were removed or detached from the surviving blastomere. Oppenheimer (1955) emphasizes that the significance of the two superficially similar experiments lay not in the tests themselves but in the investigators and their approach. She states that, "Roux interpreted the egg for the first time as a mechanism mechanically analyzable by outside interference; Driesch envisioned it as ruled by an entelchy as spiritual as any deus ex machina must be."

Spemann's constriction experiments demonstrated that there was no parcelling out of determinants from the nuclei (as proposed by Weismann) to account for differentiation at least during early stages of development of the frog.

Vogt's experiments in demonstrating the orderly movements of the outer cells of the early gastrula and final mapping of presumptive anlagen by use of vital dyes is too well known to discuss in detail here. The ensuing transplantation experiments to determine the degree of determination which could be detected within a tissue at a given time in development are perhaps best exemplified by the work of Spemann (1938) and others on the relationship of the optic evagination and the development of the lens.

The concept of induction has stimulated a considerable amount of

study, but the results to day appear to be limited to descriptions of morphological changes within proposed induction systems rather than being directed to the solution of more basic problems of molecules, molecular patterns, and molecular movements responsible for morphogenetic processes.

Experimental embryology is a rather broad field which utilizes diverse methods of study. Whether the technique be tissue culture, transplantation, surgical intervention, or teratology, the goals are: first, to come to grips with the more fundamental issues of minimal embryonic requirements for normal development, namely the interrelationship of differentiating units; second, to obtain a sharper definition of the role of genes in determining the various phases of development; and ultimately to consummate the correspondence of biochemical entities - molecules, cycles, progressions - with differentiation and the nucleo-cytoplasmic interactions.

#### Experimental Studies on Congenital Malformations

Wilson (1959) credits Saint-Hilaire (1820) with the first experimental efforts to produce congenital defects. Following St. Hilaire was Dareste, who, in 1877, proposed that abnormal development is associated with alterations imposed on the normal rate of development. In 1909 Stockard exposed fertilized ova of Fundulus heteroclitus to varying concentrations of salts in sea water and found that by increasing the amount of magnesium he could produce a high frequency of cycloptics in what he called his "magnesium embryos". He identified his experiment as the first to produce vertebrate monstrosities by use of chemical substances. Stockard

agreed with Dareste that the key to congenital abnormalities was to be found in developmental arrest which might result from an "interaction between inherent tendencies contained within the egg substance itself and the external conditions which surround and act upon this substance."

Loeb (1911) studied the fate of Fundulus eggs which had been placed into distilled water rather than salt water for development. While he did not report on abnormalities in structure, he did observe the effects of varying the environment upon survival.

Fischel (1912) had observed reports of Loeb's studies and noted that Roux's concept of complete self-differentiation by the egg was untenable, for the egg could not develop in a vacuum. In addition to commenting on the effects of temperature and radiation on ova and tissues he observed "Dies gilt wohl auch für den Gaswechsel. Den es ist festgestellt worden, dass verschiedene Eiarten gegenüber Sauerstoffzufuhr, bzw Sauerstoffmangel sehr verschieden empfindlich sind." Fischel emphasized the importance of considering abnormalities of human embryos as experiments by nature which can be compared with results of animal experimentation as a means of learning more about the development of the human organism.

During the last forty years teratologists have been seizing upon an ever increasing array of tools with which to modify the normal course of morphogenesis. The main avenues of approach have been directed toward alteration of germ plasm prior to fertilization, direct insult upon the embryo or indirect assault on the embryo through the maternal organism. The methods employed include use of chemicals which may affect osmotic

balance, serve as metabolic antagonists, or have a direct toxic effect on the tissues. Dietary studies have included starvation and hypo- and hypervitaminoses. Trypan blue has been used by a large number of investigators. Insulin, cortisone, and other endocrine substances have been tested for teratologic potentialities. Reduction in available oxygen has had teratologic effects under both atmospheric pressure and in partial vacuum. Exposure of experimental animals to alterations in temperature has also resulted in the production of anomalies. Irradiation in the form of x-ray and from unstable isotopes has been a tool for the study of developmental processes. A common denominator to all of these methods seems to be the mystery of exactly what the teratogenic mechanism is. Generally a wide variety of tissues are affected by each teratogen. Because of the orientation of the work in this paper, however, more attention will be paid to mention of abnormalities of the urogenital system rather than to a complete listing of defects reported by each author.

#### Nutritional deficiencies

Wilson (1959) lists ten maternal nutritional deficiencies which have been used to cause malformations. Kalter and Warkany (1959) credit Hale (1935) with the first controlled experiment in mammalian teratology, when, by feeding pigs on a vitamin A deficient diet, he was able to produce anophthalmos in the offspring. Included in the many reports since that date are those of Warkany and Schraffenberger (1944) and Wilson and Barch (1949) describing the abnormalities in litters of strains of rats born of mothers fed on rations deficient in vitamin A. Miller, Woolam,

and Lamming (1954) described congenital hydrocephalus in rabbits fed on a similar regimen.

Warkany (1954) reports that 53% of rats of vitamin A deficient mothers were abnormal with 90% having ocular and urogenital anomalies.

Abnormal embryos induced by maternal vitamin E deficiency have been reported by Cheng, Chang, and Bairnson (1957). A single dose of d,l-alpha tocopherol acetate administered to the vitamin E deficient rats during the period from day 4 to 8 was adequate to protect the young but if identical therapy was delayed until day 9,10,11, or 12 of gestation there were large numbers of young which showed a multiplicity of congenital abnormalities. Thirty-seven percent of the live embryos showed one or more kinds of malformations. "A study of the gross changes in the external form reveals that as early as the 11th day of gestation the abnormal embryos show a retardation in development." Included in the list of abnormalities were exencephalus, umbilical hernia, scoliosis, club feet, hare lip, receding maxillae, edema, hydrocephalus, webbed front feet, ectocardia, anencephalus, and kinked tail.

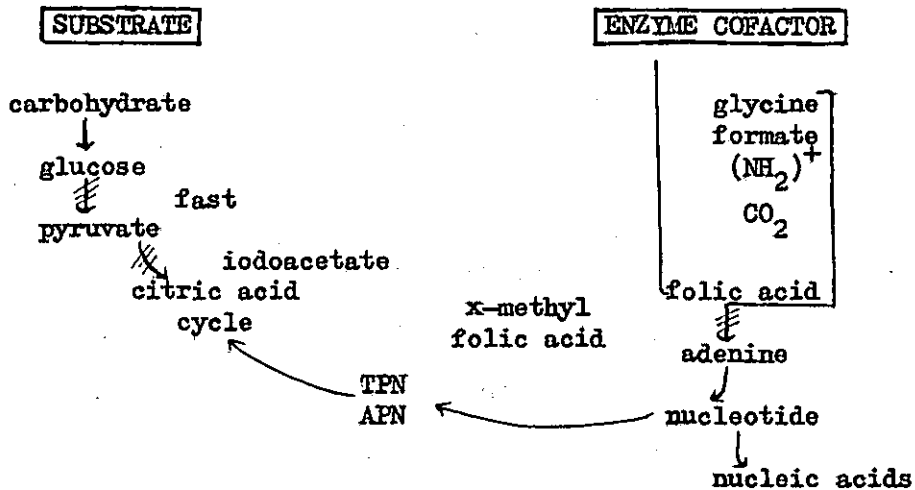
Kalter and Warkany (1959) listed other avitaminoses which result in abnormal development in experimental animals. Deficiencies of riboflavin, folic acid, and pantothenic acid are included in the itemization. The deficiencies have been accomplished either by removal of substances from the diet or by utilization of metabolic antagonists. Wilson (1959) added vitamin B<sub>12</sub>, thiamine, biotin, and vitamin D to the list of deficiencies which have resulted in congenital defects.

Starvation

Runner (1959 a,b) described abnormalities of mice which were from mothers that had been starved during their pregnancy. By varying the degree of starvation, Runner observed that certain foods had a sparing effect on the embryo. The normal rate of abnormalities for the strain of mice used was 2%. Complete fasting during the 9th day of gestation resulted in 22% of the young having abnormalities. Not only did the 9th day fasting result in increased frequency of defects but there was also an increase in resorption sites. Runner observed:

Four teratogenic agents (insulin, iodoacetate, x-methyl folic acid and fasting) produced similar abnormalities of development. ... Administration of nutrients during the fasting period and use of teratogenic agents have provided circumstantial evidence that carbohydrate metabolism may be selectively critical for morphogenesis of embryonic neural tube and for differentiation of precartilaginous mesenchyme.

On the basis of this observation, Runner (1959 b) suggested the following scheme as a possible way in which the experimental conditions described may be involved in teratogenesis:



Fasting reduces the food available to the embryo for the embryo itself has no apparent food reserves. This results in a reduction of the glucose available for conversion to pyruvate. Iodoacetate is seen to interfere with the conversion of pyruvate through the metabolic pathway into the citric acid cycle. Folic acid antagonist, x-methyl folic acid, is inadequate to serve as the enzyme cofactor responsible for participating in the conversion of certain substances ordinarily available for use in energy transfer systems.

Runner (1959a) also worked with the combined effects of hypoxia, starvation, trypan blue, x-irradiation, and various anti-metabolites on morphogenesis of the axial skeleton. He combined five hours of hypoxia with fasting on the 9th day and obtained a 25% increase in abnormalities over hypoxia alone. He noted that the mode for abnormalities of the vertebral column was located between the 5th and 6th thoracic vertebrae. In some cases the mode shifted with combination of starving with other factors. Associated abnormalities include cranioschisis, rib abnormalities, and limb defects. The individuality of treatment defects were reduced or disappeared on combination with starvation.

#### Trypan blue

Trypan blue has been used by a number of investigators concerned with embryological development and was first reported in this connection by Gilman and Gilman in 1948 (Wilson, 1959). Hamburg (1954) used trypan blue as a teratogenic agent to see if results obtained on exposure of normal mice would resemble those observed as a result of gene mutation.

Investigators in physiological genetics have been using teratogens to aid in understanding the biochemical actions and morphogenetic factors controlled by genetic mutations. The majority of malformations fall into two categories in Hamburg's work; "a. abnormalities of the anterior region, e.g. failure of the head fold to close, ... resembling the mutation pseudoencephaly, and b. abnormalities of the posterior axis, such as kinky, curly, short, and constricted tails." In a comparison of results with those described by Bonnevie on genetically determined pseudoencephaly and with Sd and brachyury mice for the caudal abnormalities, Hamburg noted some differences and similarities. The absence of notochord seen in brachyury was very rare in trypan blue treated mice but the blebs, twisting and folding of the neural tube were similar in the two instances. The tail developed normally within ten days but subsequently there occurred a breakdown of the structure with associated hematoma. This was characteristic of both Sd and the trypan blue treated mice. The mechanism of action of the trypan blue upon the embryo was considered by Hamburg to be indirect.

Fox and Goss (1957) studied malformation of the heart and great vessels produced in rat fetuses by the use of trypan blue. Fern (1958) investigated the effects of the same agent on hamster development. The abnormalities recorded were limited to those of the cephalic-axial region and included hydrocephalus, encephalocoeles, and exencephaly. The first hydrocephalic to be noted in the animal colony occurred in nine of a litter of a control animal.

Beaudoin, Allan, and Wilson (1958) noted that theories on the mechanism of trypan blue induced anomalies generally fall into three categories: (1) changes in maternal metabolism which result in secondary effects on the embryo, (2) blockage of placental transfer, or (3) direct action on the embryo. By using the chick embryo they eliminated the requirement of action being through maternal and placental structures or the metabolism of these. By noting similar results when the dye was placed in the yolk sac and directly underneath the blastoderm they were able to determine that action in the chick was directly upon the embryo. The abnormalities produced involved the neural, skeletal, and digestive systems.

Langman and van Drunen (1959) investigated the effects of trypan blue on rabbit embryos to see if doses which produced malformations in the embryos had any influence on maternal proteins. As had been noted by other investigators, the embryos appeared to be completely free of trypan blue although 17% of the embryos were malformed. The results showed that:

During normal pregnancy there is a significant trend toward a decrease in total serum protein, serum albumin, alpha and beta globulin. Injection of a one percent trypan blue solution before and during pregnancy disturbs the normal proportion of serum proteins. A significant increase of total protein, albumin, alpha and beta globulins, occurs during the first ten days of pregnancy in the treated animals. The disturbance is most serious during the time when differentiation of the major organ systems in the rabbit takes place.

Wilson, Beaudoin, and Free (1959) determined that the peak of teratogenic action in their rats would occur when trypan blue was introduced during the 7th and/or 8th day. The maximum effectiveness, found to be during the 8th day, occurs just prior to morphological differentiation

(which occurs on the 9th day) of the structures affected. This time, however, might be expected to coincide with the process of chemical differentiation. The authors stated that dye administered on the 9th day or later has little or no teratogenic effect.

### Radiation

Brown (1931) gave a thorough account of abnormalities of the urogenital system which had developed in the offspring of a pair of mice that had been irradiated prior to conception. This is an instance of direct effect of radiation on the germ plasm and production of genetically controlled abnormalities.

Rugh (1954) has reported on the effects of ionizing radiation on embryonic development in the frog. The newly fertilized egg and blastula are found to be the most radiosensitive stages whereas the ovarian egg, neurula, and older tadpole are the most radioresistant. The reasons given for this are (1) the state of the chromosomes (which are highly condensed and perhaps more exposed during meiotic and mitotic development than interphase), (2) the relative potency of the blastomeres (in that with progressive divisions more sharply defined responsibilities develop), and (3) the presence of cells differentiating into vital organ primordia (with development of new molecular and cellular associations).

It can be said that any embryonic stage (excepting the tadpole) is infinitely more radiosensitive than the adult of the same species, by any criterion or estimation. This may be related to the protracted period of differentiation or the central nervous system and its accessories.

Russell (1950) exposed the maternal organism to controlled x-ray for one dose during the period from  $\frac{1}{2}$  - 13 $\frac{1}{2}$  days of gestation. The rat embryos showed abnormalities of the anus and urogenital system. His results will be considered in more detail later in the paper.

Wilson (1954) observed the relationship between differentiation and susceptibility of rat embryos to radiation. He noted general agreement that:

Proliferating cells may show any of three such reactions [to radiation]: (1) death may occur without any further cell division, (2) mitosis may be temporarily inhibited but later resumed at an approximately normal rate, or (3) subtle genic alterations (somatic mutations) may occur, but not be manifest until late by faulty differentiation of descendent cells.

In a discussion following presentation of this paper, Kimball (ibid.) observed that there would be some difficulty defending somatic mutation as a factor "because these [somatic mutations] should be induced at all stages of development not only after the eighth day."

Sikov and Noonan (1958) varied the mode of exposure to radiation by use of radiophosphorus. Their notation of an increase in incidence and severity of malformations with increased dose and dependence of malformations on the time of exposure agrees with the observations recorded in all papers read. Defects of the urogenital system were observed in this group of experimental animals.

### Cortisone

Fraser and Fainstat (1951) have treated pregnant mice with cortisone. Five groups of animals were used, only one of which was genetically heterogeneous. Each gravid animal received treatment over a four day

period; dosages were varied among the individual animals. Cleft palate was the abnormality studied because of its ease of diagnosis and suitability for statistical analysis. The incidence of the defect was significantly different among some of the groups, indicating that the susceptibility to this particular deformity as a result of exposure of the maternal organism to cortisone is tempered by the genetic composition of the experimental animals.

#### Temperature

Smith (1957) reduced the body temperature of gravid hamsters for a single exposure of 25-30 minutes or 45-48 minutes during the interval from  $\frac{1}{2}$  -  $15\frac{1}{2}$  days of gestation. Temperatures were taken to below  $0^{\circ}\text{C}$ . Thawing and reanimation were accomplished by warming the whole body by diathermy and giving artificial respiration. Normal numbers of healthy animals were found at sacrifice following exposure for thirty minutes during the  $1\frac{1}{2}$  -  $8\frac{1}{2}$  day period or after  $13\frac{1}{2}$  days of gestation. By contrast, freezing for 45 minutes during the  $1\frac{1}{2}$  -  $8\frac{1}{2}$  day period of gestation resulted in "arrested development or caused malformation of most of the fetuses." The abnormalities described include hydrocephalus, anencephaly, herniation of the brain, absence of one or both eyes, hare-lip, cleft palate, and deformities of the feet.

#### Immunological methods

Weiss (1947) reported on the results of injection of early chicks with antiserum prepared in guinea pigs against adult chicken liver, kidney, and pectoral muscle. Body weights of the injected embryos at term were

below average, but the organs against which the antisera were injected showed evidence of positive growth stimulation. A stimulating effect was identified whether antisera were used or direct transplantation of 6 day embryonic organs to the area vasculosa of 4 day embryos was effected. This instance of stimulation by tissue transplantation is to be contrasted with one phase of the work of Gluecksohn-Waelsch (1957) in which injection of tissue extract of mouse brain into female mice over a two week period before mating resulted in abnormal development of the embryonic central nervous system. Heart extract had no effect on brain formation. Neither brain nor heart extract had an effect on the heart formation. Weiss' work was stimulated by interest in the possible "gang" function of a particular intracellular molecular species in mastering the surface characteristics of the differentiating cell. The direct stimulating effect of antibody noted by Weiss seems counter to the expected depression, but he proposes that the antibody may serve as a template for elaboration of more of the master molecule under the experimental conditions. Gluecksohn-Waelsch was interested in the possible correlation of maternal antibody formation in erythroblastosis fetalis and selective congenital tissue or organ deficiencies. The one time introduction of antibody into the chick egg is not comparable to the continuing onslaught of maternal antibodies (which apparently occurred in the mouse experiments), but the opposite effects are none the less interesting.

### Hemorrhage

Wilson (1953) produced severe hemorrhagic anemia in pregnant rats by allowing blood to flow freely from the caudal vein until at least five milliliters were collected on each of three successive days beginning with day 7, 9, 11, or 13. Only when bleeding had been initiated on the 7th day was there an indication of increased anomaly formation. The 9th day was given as the time of mesoderm formation and the 10th as the beginning of organogenesis. Anomaly rate was 7.6%, a mild degree of retardation was observed in a few specimens, and a slight increase in the number of resorptions was noted. The abnormalities observed were limited to the skeleton and central nervous system.

### Hypoxia

Fischell (1912) anticipated the later use of hypoxia as a teratogen in his observation of the varying degrees of sensitivity of eggs to alterations in oxygen supply. Interest in the role of hypoxia in embryonic development has perhaps been prompted by observation that antioxidants had been used as teratogens over the years, by interest in problems associated with aviation physiology, and further by the fact that this is one of the few agents used experimentally which might conceivably play a role in the natural production of monstrosities. The rather extreme reduction of oxygen tension which must be used to produce morphologically-expressed abnormalities are not likely duplicated under natural conditions. However, failure of diffusion, inadequate vascularity, etc. could give tissue anoxia even in the presence of adequate oxygen in the environment breathed by the mother.

Ingalls (1950) reported on use of anoxia to facilitate study of congenital anomalies by the epidemiologic method. Oxygen deficiency is one factor which would enter into the epidemiologist's trilogy of interacting forces, agent, host, and environment. In addition to rubella, syphilis, and toxoplasma as three infectious agents which have been proved to cause congenital defects, Ingalls observed that he and Gordon in 1952 noted that "...metabolic as well as infectious agents ... are generally capable of causing anomalies when acting in sublethal doses at critical stages of development." Ingalls, Curley, and Prindle (1952) reported on the effects of timing and degree of anoxia as factors causing fetal deaths and congenital anomalies in the mouse. An altitude chamber had been constructed which would allow regulation of vacuum and constant replacement of humidified air. Gravid, unpedigreed mice were exposed to hypoxia at reduced atmospheric pressures equivalent to 25,000 or 27,000 feet for a period of five hours. Mice were exposed once until a total of ten had been subjected to hypoxia on each of 17 days between days 2 - 8, inclusive. The specimens were removed from the uterus on the 19th day and examined macroscopically for external and internal abnormalities. Evisceration was followed by fixation and preparation of the skeletal structures by use of alizarin red. The highest percentage of death (or resorptions) and abnormal embryos resulted from exposure to 27,000 feet for five hours during the  $7\frac{1}{2}$  -  $12\frac{1}{2}$  day interval of gestation. The type of anomaly produced was dependent upon the time of exposure. Included in the list of defects were, "in order of onset, septal defects, anencephaly, irregularities and fusions of ribs and vertebrae, cryptorchidism, cleft palate

[occasionally associated with micrognathia] and open eye." In addition to these have been retinal defects and hydrocephalus. Ingalls comments:

It is reasonable to believe that the mode of action of the low-pressure insult is primarily anoxia of both mother and progeny brought about by lowered arterial saturation and tension, resulting in low tissue oxygen in the mother and fetus and local stress of those rapidly differentiating tissues that are known to have a growing capillary supply and a high demand for gaseous interchange. Doubtless many homeostatic mechanisms and enzyme systems are ultimately impaired, and certainly capillary damage plays a role in the pathogenesis of the induced anomalies.

The role of vascular abnormalities was considered by Tedeschi and Ingalls (1956) who observed hamartomatous defects in association with cranioschisis, hemi-vertebra, rib defects, kinky tail, cleft palate, and abnormalities of the eye. The vascular abnormalities did not seem to be "stage specific" but rather reflections of a general sensitivity of the mesenchyme involved in the development of the vascular system at the time of exposure. The value of laboratory experiments in contributing to an understanding of congenital defects has been compared by Ingalls (1956) to the usefulness of the fruit fly in genetic studies.

Ingalls, Kelemen, and Curley (1957) studied the development of the inner ear of mice after a single exposure to altitudes between 27,000 and 40,000 ft. for five hours between the 9th and 18th day of gestation. The complete absence of detectable defects was thought to be related to the relative avascularity of the organ at the time of the hypoxic stress. Furthermore, the ossicular chain was considered to fall into the category of primordia and undifferentiated precursors, which along with fully differentiated structures, are relatively resistant to teratologic injury.

In view of the increasing number of investigators utilizing hypoxia to study congenital defects and to enhance expression of characteristics determined by recessive genes, Curley and Ingalls (1957) exposed mice during the 10th day of gestation to two hours of hypoxia at normal atmospheric pressure by using 6% oxygen in an atmosphere of nitrogen. A survey of skeletal defects revealed malformations of the kind obtained on exposure of 8,9, and 10 day gravid females to five hours of hypoxia at an altitude of 25,000 to 27,000 ft. The 6% oxygen is described as comparable to an altitude of 30,000 feet. Neither time, duration, nor altitude in this study were equivalent to the conditions under which Ingalls' other experiments were run.

Ingalls and Philbrook (1958) investigated the effect of reduced oxygen tension on embryogenesis of Fundulus heteroclitus and zebra fish eggs in an experiment somewhat paralleling those of Stockard (1921). Four batches of eggs were placed in sea water that had been scrubbed with nitrogen. The atmospheric pressure was then reduced to an equivalent of 60,000 feet with one test series held at 70°F and the other at 86°F. Exposure for 5 - 20 hours was required to obtain systematic production of anomalies. Anomalies included anophthalmia, microphthalmia, cyclopia, rumplessness, hydropericardium, and conjoined twins. These defects were obtained by exposure of eggs at various stages from the two-cell stage to the gastrula stage. There was some overlapping of defects among the various stages. The authors concluded that "heating and hypoxia combined registered a greater teratologic impact on the embryo than hypoxia alone."

Holland (1958) exposed two groups of gravid rats to hypoxia from the 18th to the 21st day of gestation after sham adrenalectomy of one group and bilateral adrenalectomy of the other to see if hypoxic stress might be a factor in enlargement of the fetal adrenal gland. He concluded that, "it is not certain that a relationship exists between the relatively hypoxic environment the fetus is in near term and the adrenal enlargement present in most animals at birth."

Barber (1957) in a paper entitled, "The effects of maternal hypoxia on inheritance of recessive blindness in mice", described the results of trypan blue treatment of female mice, possessing recessive genetic factors for bilateral anophthalmia, made gravid by males having dominant factors for normal eyes. Slightly less than half of the survivors had unilateral anophthalmia. She suggested that, "a teratogenic agent may create an environmental stimulus or metabolic disturbance in the uterus comparable to that controlled by a genetic complex and result in the expression of a recessive congenital trait that would otherwise be suppressed." The use of the term hypoxia in Barber's paper is apparently based upon theories that trypan blue causes excessive phagocytosis of erythrocytes in the mother or blocks placental transfer, either of which might result in hypoxia of the embryo.

Degenhardt (1954) investigated the results of short term exposure of pregnant rabbits to oxygen deficiency during the 8th to 10th day interval of gestation. He noted that the gestation period was prolonged as a result of hypoxia and that the rate of malformations increased as the duration at altitudes was increased from four hours (23% showing

malformations) to seven hours (39% showing malformations). Twenty-two and one-tenth per cent showed congenital abnormalities of the central nervous system and 38.6% showed abnormalities of the skeletal system. The abnormalities were observed only in animals which had been exposed to oxygen deficiency during the 8th to 9th days of gestation. One case of hydro-nephrosis was described. In view of the range of defects observed, Degenhardt comments, "Morphogenetisch is die Annahme berechtigt, dass die kritischen Differenzierungsphasen der genannten Organanlagen zeitlich eng koordiniert sind."

Murakami (1955) exposed mice during the 8th day of pregnancy to reduced atmospheric pressures ranging from 250 mm. Hg to 400 mm. Hg for a period of five to six hours. He noted that abnormalities of the nervous system were most abundant. One of his summary statements read, "Thus it was presumed that an air-ride in a pressurized plane for several hours may not be harmful to the development of human embryos in early stages."

Nelsen (1958) achieved low oxygen tension in his work on early chick development by using a variety of mixtures of oxygen, CO<sub>2</sub>, and nitrogen. After equilibrium had been established within the chamber, a constant flow of humidified gases was effected and temperature of the chamber held to 37.5-38°C. The eggs were exposed from the beginning of incubation for varying lengths of time to determine the sensitivity of various stages of development to abnormal proportions of gases at atmospheric pressure. Nelsen observed that the requirements of the primitive streak for oxygen increased from a low of 2% toward a high of 6% as it

developed from the beginning streak, through the broad streak, to the definitive streak, and on to the head-process stage at 20-24 hours. While these concentrations were adequate for streak development, he noted that, "individuation of the various rudiments, particularly of the head, is disturbed." As development proceeded, it was found that the oxygen requirement increased. Twelve to fourteen percent oxygen was described as permitting normal development through the first two days. He concluded that, "two levels of minimal oxygen are evident, namely, the level necessary for the development and function of the primitive streak and the level required for normal development of the individuating rudiments."

Grabowski and Paar (1958) having noted the use of extremes in hypoxia by teratologists were determined to ascertain the effects of graded doses of hypoxia on the chick. They mentioned the work of other investigators who used partial vacuum, eggs coated with impervious substances, and hypoxia at normal atmospheric pressure. Reference was also given to studies of the effects of hypoxia on amphibian embryos. Grabowski and Paar introduced the eggs to a large, warmed dessicator in which soda lime had been deposited to absorb carbon dioxide and the walls of which had been moistened to maintain a moderate degree of humidity. The oxygen content of the chamber was varied from 29.2% to 1.8% of normal; exposure was for a six hour period. In this most analytical appraisal of hypoxia, the authors concluded that oxygen concentrations of more than 75% of normal constitute a "latent range" where frequency of anomaly formation was not significant. Below 60% there was a great variety of anomalies. Susceptibility increased with age until at some point death and anomaly rates were equivalent and then the latter was surpassed.

Historical Development of Research on The  
Interrelationship of Vertebrate Kidneys

Haekel's biogenetic principle perhaps found its greatest number of adherents among students of vertebrate embryology through their familiarity with the fascinating sequence of events which occur in the development of the kidney of the higher vertebrates. The relegation of the primitive kidney (the pronephros) to adult functional status in the Agnatha only, the early larval dependence on the pronephros before assumption of its function by the mesonephros in more advanced vertebrates, and the final, cursory acknowledgement of a pronephros but dedication to even better things than the mesonephros by the amniote development of the metanephros, certainly would all lend their support to a theory of recapitulation. A more critical examination of the developmental sequence produces, on the one hand, evidence which increases one's admiration of the basic set of rules which are inviolate for perpetuation of all forms of life, and, on the other hand, causes one to smile at the details of phylogeny which are slurred over during ontogeny.

Investigations of development of the excretory system have been concentrated to a great extent on development in the amphibians and in the chick. By use of vital dyes, Vogt (Holtfreter and Hamburger, 1955) was able to trace the progression of blastula and gastrula cells into position prior to morphological differentiation. The regularity of migration enabled embryologists to prepare maps of potential nephrogenic areas long before they became either morphologically differentiated or even localized in their respective areas of development.

### The pronephros and mesonephros

Holtfreter's (Holtfreter and Hamburger, 1955) interference with normal amphibian development by placing a blastula into an abnormal environment consisting of a slightly hypertonic physiological salt solution resulted in the entomesoderm passing toward the dorsal lip but then exogastrulating rather than involuting. Within the exogastrulated area pronephric tissue developed. This teratologic approach to the study of regional potentialities certainly indicated that assumption of an abnormal position by the entomesodermal cells involved did not prevent their differentiation and further showed that their subsequent differentiation had already been subject to some regulatory factors.

However, in addition to mapping the organization of early undifferentiated embryonic tissues, an approach to the sequential and dependency relationships of organ systems was found by methods which interfered with normal developmental processes of tissues during their differentiation. The problem posed by Schreiner (1902) with regard to the interrelationship of pronephros and mesonephros has been attacked by a number of investigators. The confiscation of the pronephric duct by the mesonephric kidney under the guise of eminent domain had suggested a dependency, but this had not been experimentally proved. Machemer (1929) observed the change in competence of the posterior nephric tissue of the amphibian Triton alpestris between the neurula and the 7 - 8 mm. larval stages. Transplantation of as yet undifferentiated posterior nephrogenic tissue from an older larva to the pronephric area or to the eye socket of a neurula resulted in formation of mesonephric tubules. Machemer concluded that the mesonephric

anlage in its development is subject to at least two determinative substances: the first prepares the anlage of the neurula, the second that of the tailbud. The substance involved in the earliest determination is designated for building of the basic duct, not for the building of typical mesonephric tissue. The substance which acts later determines the potential of the tissue to form mesonephric structures. Thus the function of time in differentiation of the nephric ridge area was indicated.

Miura (1930) extirpated the pronephric duct of the frog neurula and noted that although the duct failed to regenerate, the mesonephric duct developed in the absence of the pronephric duct. If there was partial extirpation of the pronephros with subsequent dilatation of the pronephric tubules, the development of the mesonephros was hastened. Shimasaki (1930b) observed successful development of the mesonephros in situ and in transplants either with or without association with the pronephric duct. Transplants did develop more luxuriantly in the presence of a pronephric duct but he concluded that the pronephros has no directing influence on the development of the mesonephros in the frog larva. Although primary mesonephric tubules develop in the absence of the pronephric duct, it was observed that the secondary and tertiary tubules do not develop under these circumstances. Bilateral extirpation of the pronephros did not affect the development of the mesonephros (Shimasaki, 1930a), but tubule development was more normal if preceded by development of the mesonephric duct. This is related to a developmental history in which secondary or tertiary tubules fail to develop in the absence of the Wolffian duct and not due to mechanical factors (Shimasaki, 1930b).

Humphrey (1928) explored the developmental potencies of the intermediate mesoderm of Amblystoma in germ stages 21-34 when transplanted into ventrolateral sites in other embryos and found normal development of mesonephric tubules independent of the Wolffian duct.

Boyden (1927) approached the relationship of the mesonephric ducts and tubules by interrupting the normal course of development in the chick. When the growing tip of the mesonephric duct was damaged by cauterization, the duct failed to develop posterior to that point. Mesonephric tubules did not develop caudally nor did a ureter form, although the metanephric condensation was not prevented from appearing.

Grünwald (1937) found that the mesonephric tissue would differentiate only in the presence of the Wolffian duct. His second observation dealt with the fact that lesions of the posterior Wolffian duct resulted in failure of the ureteric bud to develop and subsequent failure of metanephros formation. A comparison was made of the dependence of the metanephric tissue on the ureter and of the dependence of the mesonephric tubules on the mesonephric duct. He further observed that development of the Mullerian duct in humans follows the lead of the Wolffian duct and will not develop significantly in its absence. The gonads were recognized as being independent of renal development. Grünwald reviewed the literature and noted that Hunt had transplanted parts of chick embryos to the the chorioallantoic membrane and obtained full development of the mesonephros, but observed that the transplant had been made after development of the mesonephric duct had begun in the donor. Grünwald (1942) observed that introduction of normally non-nephrogenic mesenchyme into the path of

the developing mesonephric duct generally either prevented further development of the duct or caused a deviation of the duct from its normal course with premature termination or resumption of its course after passing the obstacle. The Wolffian duct did not elicit nephron formation in non-nephrogenous tissue, but contrary to Gruenwald's and Boyden's earlier reports, Gruenwald noted that mesonephric tubules, more or less vestigial in form, can develop in the absence of the mesonephric duct. He observed the formation of mesonephric tubules adjacent to a ganglion and suggested that nervous tissue might be capable of inducing mesonephric tubule formation in the absence of the normal physiological inductor. In one instance the Wolffian duct induced formation of mesonephric tubules in the metanephric blastema. On the basis of this observation, Gruenwald concluded that there is only "one nephrogenic potency which responds by mesonephros formation to the stimulation by the Wolffian duct, and by metanephros differentiation to induction by the ureteric bud." In two cases the Wolffian duct had ended on the surface of the embryo, apparently diverted from the normal path by the grafts. No tubules were found near these ducts.

Hoadley (1925) investigated the development of the nephrogenic tissue of the chick blastoderm. The nephrogenic tissue lies posterior to the primitive knot in the anterior two-thirds of the primitive streak. Because of the sequence of formation of secretory tubules, glomeruli and, finally, the duct, Hoadley concluded that the pronephros and mesonephros are homologous and that the more specific differentiation in the grafted tissue was a function of age. This conclusion coincides with that of Machemer (1929) suggesting a similarity in development of the nephrogenous tissue of

amphibians and avians studied in that the difference in determination of tissue into mesonephros rather than pronephros is a function of time during which differentiation can take place.

Bremer (1916) noted the considerable difference between mesonephric development in different classes of animals. He noted that, while well-endowed mesonephroi were developing glomeruli in such an animal as the pig, Wolffian bodies of the rat, for example, were degenerating. The degeneration occurred prior to functional differentiation of the metanephros and without formation of glomeruli. Bremer observed that "there appears in the rat...at about the time of normal development of glomeruli in other groups the development of glomerulus-like structures in the placenta — that the placental-endometrial relationship is identical with that of mesonephric glomeruli and capsules."

Gersh (1937) described the correlation of structure and function in the developing mesonephros and metanephros in rabbit, cat, opossum, pig, and chick. He stated, however, that "no relationship is indicated between structure of the allantoic sac or placenta and the time of degeneration of the mesonephros" in these animals.

#### The metanephros

Schreiner (1902) reviewed the literature of the nineteenth century which had been devoted to a study of development of the amniote kidney and stated that the advances which were made in understanding relationships within the urogenital system were slow, largely because of limitations in technique. He mentioned Valentin's work with the human in 1835 and Reichert's

investigation in 1840, in which both observed formation of the ureters from the ureteric bud. Remak (1855, cf. Schreiner) reported the metanephros of higher vertebrates as being an outgrowth of the cloaca. Kolliker (1861, cf. Schreiner) in studying the human and other higher vertebrates observed the club-shaped termination of the ureter in the metanephric area and reported that the inner portion formed the calyces and nephrons while the outer portions formed the glomeruli. Schreiner also recorded Kupffer's conclusion that the outer layer of the metanephric blastema came to form the nephrons "unabhängig von dem Nierenbecken und den Zellen der inneren Zone." Sedgewick (ibid.) described the development of the metanephric blastema in the chick and observed that the kidney blastema collects around the end of the ureter and from these collections the kidney tubules grow out.

In his monumental work, Schreiner described the embryological development of the excretory systems of the reptile, bird, and mammal. His realization of the intimate relationship of the Wolffian duct of the mesonephros and the development of the metanephros after contact with the ureteric bud led him to conclude:

Der Unterschied zwischen einem Vornieren und einem Urnierenkanalchen ist darum nicht nur in der Materialverwendung, sondern auch in dem Material selbst gelegen. Dieses Verhalten bedingt, wie mir scheint, einen viel tieferen Unterschied zwischen Vorniere und Urniere als zwischen letzterer und Nachniere."

Pohlman (1905) prepared a paper entitled, "Abnormalities of the kidney and ureter dependent on the development of the renal bud." Based largely on material studied in Mall's collection and autopsies which he had performed himself, Pohlman prepared a diagram of various anomalies of

of the kidney and ureter which were dependent upon development of the renal bud. The combination of abnormalities included, however, a broader range, for his illustrations also depict atypical renal positions due to failure of normal ascent of the kidney to its usual position.

Fischel (1912), in following the studies of Schreiner, conjectured that just as the pronephros of the human is important only as a rudiment, and the mesonephros has a similar fate, perhaps the significance of the latter rests with a causal relationship between the ureteric bud and the nephrogenic tissue of the metanephros. Fischel noted from his own study that failure of the ureteric bud invariably results in failure of differentiation of the metanephros. He considered this a mutual dependency such that neither anlage can differentiate without reference to the other. The possibility of the metanephric tissue directing ascent of the ureteric bud was mentioned.

Boyden (1927) had observed that "neither the mesonephric nor metanephric tubules develop in the absence of the Wolffian duct from territory adjacent to the appropriate nephrogenic tissue."

Gruber (1929) reviewed much of the literature on abnormal development of the kidney and ureter and, in mentioning Chwalla's catalogue of more than 120 human dissections, noted the similarity in excretory anomalies of humans and animals. He referred to von Gierke's description of an interrelationship of ureter and post cardinal vein and suggested that the latter may get in the way and require an unusual path of development for the ureter.

In 1931 Brown reported on the histological study of a strain of mice derived from a previously x-rayed pair (Bagg, 1925) in which there was a high incidence of abnormalities of the kidneys. Her observations indicated that failure of the ureter to gain access to the metanephric blastema was the immediate cause for metanephric agenesis. More basically involved was anomalous development of certain components of the vascular bed. She observed that in the mouse a correlation appears to exist between the metanephric blastema and ureteric bud in that the amount of rudimentary blastema present is in proportion to the degree of ureter development. She states, "I am, therefore, inclined to disagree with Pohlman ('05) that the Wolffian anlage is completely responsible for stimulating kidney development." If either of the anlagen is retarded, there may well be failure in kidney formation.

Grünwald (1937) reviewed Willier's transplantation of metanephric tissue to the chorioallantois and its subsequent development. The transplants successfully differentiated into metanephros only when removed from five day embryos, by which time the ureteric bud has grown up to the renal anlage. It will be recalled that in his own experiments Grünwald noted failure of the ureteric bud to form in chicks and stated that the metanephros was dependent upon the caudal end of the Wolffian duct just as is the mesonephric tissue dependent upon the duct.

Grusenwald (1942) had observed formation of mesonephric tubules in the metanephric blastema by induction associated with the Wolffian duct and referred to a difference in behavior when the mesonephric and metanephric blastemas are unstimulated by their "physiologic inductors."

Gluecksohn-Schoenheimer (1945) described a simple dominant gene, Sd, as responsible for absence or reduction or one or both kidneys in heterozygote mice. In her dissections it was revealed that agenesis was due to failure of the ureteric bud to form or to reach the metanephric area. Renal hypoplasia occurred in many instances but the ureter was always present under these circumstances. The same author presented additional information on the excretory system of Sd mice and observed that development was normal up to the time of ureter formation from the mesonephric ducts. It is interesting to note that Gluecksohn-Schoenheimer (1949) observed that whatever kidney develops in Sd+ mice, there is a normal quantitative relationship of medullary and cortical tissue. This relationship adds weight to the observations of Brown (1931).

Potter (1946) had surveyed results of over 5,000 autopsies of fetuses and newborns and recorded twenty cases of renal agenesis. It is interesting to read her comment that "whatever agent prevents out-growth of the ureteral bud from the Wolffian duct inhibits the caudal extension of the Mullerian duct. The Wolffian ducts are not intrinsically altered and in all male fetuses..." the reproductive and accessory glands are normal. Auer (1947) contributed a case of bilateral agenesis associated with rudimentary Wolffian bodies and ducts in a 15.5 mm. crown-rump human embryo. He observed abnormalities in the vascularization of mesonephric tissue and that the relatively few mesonephric glomeruli were atypical. A surprising statement is that "as vessels play a rather passive role in ontogeny, it is believed that they are not

responsible for these extensive anomalies."

Additional information about normal relationships of nephrogenic structures has been obtained by experimental teratologists. Wilson and Warkany (1948) reported a high incidence of malformations of the genitourinary tract in mice born of mothers supported on a diet deficient in vitamin A. The defects were associated with displaced or absent kidneys. Ureters were always present, rarely malformed, "but with great regularity they were found to terminate at abnormal sites in the lower G.U. tract."

In the course of discussion of bilateral renal agenesis, Macht (1950) referred to Madisson's theory that a defect in the germ plasm might cause abnormality in the development of an organ such as the kidney. Madisson's suggestion came after he had noted bilateral renal agenesis in siblings. No similar reports were known to Macht.

Orsini (1952) described a piebald line of hamster in which there was an association of growth retardation, aplasia of the female urinogenital tract and the degree of piebaldness. The degree of abnormality ranged from complete absence of a kidney to failure of development of a portion of the uterine horn. Although genetic determination of renal agenesis has not been identified in humans, genetic factors have thus been found to affect the frequency of a renal anomaly in development of other mammals.

Carpentier and Potter (1959) noted a combination of abnormalities which were frequently associated with bilateral renal agenesis. They further noted that there was a 3:1 ratio of bilateral agenesis between males and females in the human.

Grobstein and colleagues have undertaken a fascinating study of inductive relationships in embryonic development. In 1953 Grobstein isolated very early rudiments of submandibular gland bud epithelium (13 day) and kidney bud epithelium (11 day) from mice. Both buds were already within their respective mesenchymal condensations. The mesenchymal components were enzymatically separated from the associated epithelial tissue. The mesenchymal masses were then subdivided and cultured circumferentially about the epithelial buds. In all cases the mesenchyme condensed about the epithelial tissues. Kidney mesenchyme began differentiating into tubules about the ureter and acini developed within the submandibular capsular mesenchyme. Kidney mesenchyme also differentiated into tubules after aggregation about submandibular epithelium. Grobstein had found that dorsal neural tube of the mouse embryo would induce tubulation of metanephric mesenchyme in culture (recall Gruenwald's observations in 1942). By placing metanephric mesenchyme on one side and dorsal spinal cord on the other side of Millipore filters, he studied the induction of kidney tubule formation. Three filters of known porosity and thickness were used. After culturing, the filter preparations were examined with the light microscope, phase microscope, and then the electron microscope. The possibility of cytoplasmic contact between tissues on the two sides of the finest filters (i.e. with least porosity) "appears to be negligible. It is concluded that inductive activity in this system is not dependent upon cytoplasmic contact and hence is resident in materials which are at least potentially extracytoplasmic."

It has been noted that interest in embryonic development has existed for some time. Studies of morphogenesis were at first accomplished in a surprisingly systematic manner such as is exemplified by Aristotle's examination of the avian egg, but accuracy of observation was subjected to teleological interpretation. The authoritarianism of Aristotle unfortunately persisted through the centuries with the apparent unwillingness of independent observers to break through to really significant principles of development. The emancipation from spontaneous generation foreshadowed by Harvey, the stimulation produced by debate between epigeneticists and preformists, and the experimental approaches to problems of development begun by Roux and Spemann have opened the field for remarkable achievements in the technological revolution of our time. It is against this background that I present a consideration of the results obtained by use of hypoxia as a teratogen in understanding the normal development of the excretory system of an albino strain of Mesocricetus auratus.

## MATERIALS AND METHODS

All animals used were non-inbred albino hamsters (Mesocricetus auratus) supplied by Bio-Research Consultants (Cambridge, Mass.) or were the offspring of matings between albinos obtained from that source. They were maintained on Purina Lab Chow pellets ad libitum without supplementation.

The estrous cycles were followed by vaginal lavage as described by Ward (1946). The breeding procedures were those recommended by Whitney (1957). Proestrous females were placed in individual cages with albino males in the late afternoon, and the following morning the animals were separated and a vaginal lavage performed with a medicine dropper and saline. The material obtained was examined unstained in Boerner slides at 100 magnifications to detect the presence of spermatozoa. Vaginal plugs were not found consistently so could not be relied upon for evidence of copulation. Estimates of the age of the embryos was based on the work of Ward who found that the ovulatory peak of the golden hamster occurred at approximately 1 a.m. and that the period of ovulation was approximately from 12:00 midnight to 2 a.m. Since ovulation in the hamster has been shown not to depend on copulation, it was determined to use the hour of 12:00 midnight as a convenient basis for determining age. This method, although arbitrary, would seem more reliable than use of copulation time since coitus has been observed during the period from 2 p.m. in the afternoon of proestrous until 9 a.m. in the day of estrous.

An altitude chamber was improvised by using two large sized desiccating jars, a U-tube mercury manometer, a perfusion jar, T-shaped connectors, a Welch Duo-Seal vacuum pump, and a supply of compressed air from the laboratory line. The arrangement of the components is illustrated in figure 1. Compressed air was bubbled through distilled water contained in a perfusion bottle. The valve was set to assure a flow of more than two liters per minute, as determined by displacement of water at atmospheric pressure, into the chamber system. The moistened air then passed through a T-tube to each of the chambers. The inlet for the chambers was located at the level of the lip of the jars. The animals were supported on a wire screen resting on the shelf supports. The exhaust was drawn from the bottom of the chambers to a T-tube from which a single hose passed to a manifold adjacent to a trap. A second line, which was open to the atmosphere at the beginning of a chamber run, was also connected to the manifold. The speed at which vacuum was achieved and the degree of vacuum desired were controlled by regulating the amount of air admitted through the second line. Because of the relatively large volume of air passing through the pump, the exhaust was led from the pump to a Robert's filter pump so that the objectionable oil mist produced would not gain access to the laboratory.

A total of 79 females was used in the project; 49 were used in preliminary screening and the remaining 30 animals for final study.

Gravid females were exposed to decreased atmospheric pressure at various times during the gestation period, limiting the exposure to a single encounter on day 7,8,9, or 10 for a duration of 5,7½ or 10 hours.

Two hundred seventy-three embryos derived from 38 females were examined grossly and under low power for abnormalities of the excretory system. The embryos were sacrificed during the 15th day of gestation. Examination for excretory defects was limited to those involving the kidney and ureters. The findings recorded from this group of animals were compared with those obtained by an examination of 103 control embryos of the same age.

Twenty-four females were subsequently exposed to a reduced atmospheric pressure for ten hours during the interval of embryonic life theoretically determined to be from 8 days, 1½ hours to 8 days, 11½ hours. The pregnancy of these animals was interrupted to supply embryos which were of developmental ages from 8 days, 14 hours to 12 days, 14 hours. One hundred and five of these embryos were compared with controls of similar age obtained from litters of six females and the normal hamster embryos described by Graves (1945), Ortiz (1945), and Boyer (1948,1953).

The animals were weighed and placed in the altitude chamber either alone or with one other animal in the same compartment. Pressure was reduced at the rate of 2 cm. Hg per minute until an atmospheric pressure of 220 mm. Hg. (equivalent to approximately 30,500 ft. altitude) was obtained. The system was sufficiently stable to maintain pressure within a range of 200 to 240 mm. Hg. This range is equivalent to an oxygen pressure of 40.5 to 48.6 mm. Hg or of 25.5 to 30.6% of normal atmospheric oxygen. Pressure was calculated according to the following formula:

$$ppO_2 = 0.209(P - 23.8)$$

where P = total barometric pressure in mm. Hg  
 0.209 = fraction of oxygen in air, and  
 23.8 = vapor pressure of water at 25°C, in mm. Hg

Percentage of oxygen at experimental level relative to that at sea level is determined by the equation:

$$O_2 \text{ \% of normal} = \frac{ppO_2 \text{ at experimental level}}{ppO_2 \text{ at sea level}} \times 100$$

Descent was accomplished at the same rate as ascent. Temperature of the chamber was slightly higher than room temperature. The room was cooled by an air conditioner and temperatures within the chamber remained within the range from 23°C to 27°C.

The animals were sacrificed by exposure to ether. They were opened by a ventral incision, cornua were exposed, implantation sites were counted and ovaries and uteri excised. The number of corpora lutea was determined. The abdominal cavity of each fifteen day embryo was punctured at two points and the embryos fixed in 70% alcohol. These embryos were fixed for 48 hours, dissected, and studied with the assistance of a low power dissecting microscope. The uteri containing the embryos to be studied for development at 8,9,10,11, and 12 day stages were cut between implantation sites and the sections were placed into Tellyesniczky's alcohol-formalin-acetic acid fixative for 48 hours. The embryos were then dissected from the uteri and stored in 70% alcohol until processed by the paraffin technique. Serial transverse sections were cut through the entire smaller embryos and posterior half of each of the larger embryos at 10 micra. The anterior half of each of the older

embryos was sectioned at 14 micra. Sections were processed through hematoxylin and eosin after passing through Regaud's lacquer to minimize loss of sections. Examination of sections was accomplished at magnifications of 22.3X to 440X.

## RESULTS

Preliminary Experiments to Obtain Optimal Exposure Durations and Gestational Times

The results of five-hour exposures of gravid female hamsters to hypoxia during the morning of days 7,8,9, and 10 of gestation are recorded in table 1. The survival statistics are given for each age group of experimental animals and for the control embryos. The number of embryos with abnormalities of the kidneys or ureters are tabulated for each group.

TABLE 1

FIVE HOUR EXPOSURE OF GRAVID FEMALE HAMSTERS  
TO 220 ± 20 MM. HG ATMOSPHERE

Gestational period	Number of animals	Corpora lutea	Implantations	Resorptions	Abnormalities
7th day	6	68	65	2	1
8th day	5	62	56	13	9
9th day	5	68	31	19	0
10th day	4	59	54	18	0
Controls	10	122	104	1	0

Abnormalities of the excretory system were found in 21% of the embryos which survived maternal exposure to hypoxia on the eighth day (9 abnormalities out of 56 implantations). However, a single abnormality only (1.6%) was found on the seventh day of gestation.

Using animals on the 8th day of gestation, a second series of experiments was conducted to determine the duration of hypoxia, up to ten hours, which would yield the highest percentage of abnormalities without too great a loss of litters. The results of these experiments are recorded in table 2 and include the effect on individual litters rather than on groups. Throughout the paper the term normal is used with regard to the excretory system only

TABLE 2  
FIVE-TEN HOUR EXPOSURE OF INDIVIDUAL HAMSTERS TO HYPOXIA  
DURING THE EIGHTH DAY OF GESTATION

5 Hour Exposure							
Start of Exposure	Animal number	Corpora lutea	Resorbing embryos	Normals		Abnormals	
				♀	♂	♀	♂
1:30 a.m.	94	13	0	3	6	1	0
	95	15	1	6	4	1	0
	30	15	1	4	3	3	2
	25	10	3	4	2	1	0
	3	10	8	0	2	0	0
	38	13	1	5	4	0	2
10:30 a.m.	16	14	0	-10-		2	0
1:30 p.m.	115	12	0	3	3	1	0
	74	13	6	0	0	0	1
7:30 p.m.	102	16	1	5	3	1	2
	93	14	0	5	6	0	0
7½ Hour Exposure							
7:30 a.m.	36	12	5	1	3	0	1
	90		not gravid				
	96	13	4	1	3	1	1
	99	13	0	7	6	0	1
10 Hour Exposure							
1:30 a.m.	92	12	9	1	0	2	2
	113	12	8	0	0	0	1
	118	14	9	2	1	1	1
	120	13	5	3	0	2	2
7:30 a.m.	97	8	3	0	1	2	1
	103	12	4	0	0	0	0
	108	13	2	6	2	0	3
	112	10	10	0	0	0	0

The individual results of table 2 are totaled and summarized in table 3, indicating the relative percentages of reabsorbing embryos, normal embryos, and abnormal specimens. Inspection of the data shows that a 10 hour exposure to hypoxia produces a significantly greater degree of defective embryos at 8 days than exposure of 5 or 7½ hours.

TABLE 3

TOTALS AND PERCENTAGES OF IMPLANTS DEAD OR RESORBING, NORMAL AND DEFECTIVE EMBRYOS OF MOTHERS EXPOSED TO GRADED DOSES OF HYPOXIA DURING THE EIGHTH DAY OF GESTATION

Duration of exposure	Number of gravid females	Implants					
		Resorptions		Normals		Abnormals	
		No.	%	No.	%	No.	%
5 hours	11	19	16.6	78	68.5	17	14.9
7½ hours	3	9	26.4	21	61.8	4	11.8
10 hours	8	50	60.2	16	19.3	17	20.3
Controls	10	1		103		0	

The frequency of abnormalities based on sex and the side on which the defect occurred is entered in table 4. Each abnormality is recorded in its own right. From a total of 153 embryos which survived the hypoxic insult, 20 males and 18 females were found to have the following grossly observable defects of the kidney and/or ureters; agenesis, hypoplasia, displacement, or distension.

Abnormalities of the excretory system were varied in individual animals as illustrated by the following specific examples.

In embryo 30K both kidneys were to the left of the midline. The right kidney was fused with the caudo-medial aspect of the left kidney capsule. Each kidney possessed a ureter but although these were fused

just prior to entering the kidney substance, they arose from the usual locations on the bladder.

TABLE 4

THE NATURE OF GROSS URINARY DEFECTS IN EMBRYOS OF HAMSTERS EXPOSED TO GRADED DOSES OF HYPOXIA (220 MM. Hg) ON THE EIGHTH DAY OF GESTATION

Kidney abnormalities	Side		Total	Sex	
	Left	Right		Male	Female
Agenesis	13	8	21	10	11
Hypoplasia	3	1	4	3	1
Displacement	3	4	7	5	2
Ureter abnormalities					
Agenesis	13	7	20	10	10
Distension	8	9	17	9	8
Displacement	5	4	9	7	2
Ratios, Left : Right	45 : 33			44 : 34	

The hilus of the right kidney of embryo 38J was directed caudally with the ureter passing to the testis. The left kidney was absent.

The only kidney present in embryo 16E was located on the left side. Although drained by a single ureter, it was an elongated structure with a concavity directly opposite the hilus so that the organ looked bilobed.

Embryo 113A presented kidneys fused at their craniomedial margins. The ureters were fused for a short distance between the kidneys and then separated to approach the bladder in normal fashion.

The ureters were often distended (hydroureter) and, in several instances, were also kinked. These conditions were observed both uni- and bilaterally. Figures 2-6 include selected gross dissections of normal and test embryos from this series.

It was decided to use a ten hour exposure to 220 mm. Hg atmospheric on the eighth day of gestation between the hours of 1:30 a.m. and 11:30 a.m. to obtain embryos for microscopic study. This selection was based on the approximately 50% frequency of abnormalities of the urinary system among survivors in the preliminary study. Complete litters were removed by Caesarean section on the 9th, 10th, 11th, and 12th day of gestation and examined in serial cross sections for abnormalities. Because some of the abnormalities observed in the older specimens could not be observed in less developed embryos, the results are tabulated separately for each developmental age

#### Histological Findings After Ten Hours of Maternal Hypoxia at Eight Days of Gestation

At nine days, the control embryos show a mesonephric duct which is patent for its entire length to its termination adjacent to the cloaca. Condensations of mesenchyme can be seen just ventral to the mesonephric ducts and posterior cardinal veins. The more anterior clusters have convoluted and begun to cavitate (fig. 7). In a few instances the first tubules have attached to the duct. The mesonephric condensations continue posteriorly almost to the border of the dense metanephric masses. The mesonephric ducts approach the metanephric blastemas laterally and then pass ventromedially to approach the cloaca posterior to the origin of

the umbilical arteries. The right umbilical artery is formed by the fusion of two buds from the aorta. A part of the right metanephric condensation or mesonephric duct is often completely surrounded by these vessels. The ureteric buds develop from the mesonephric ducts as the latter pass ventromedially to the cloaca. Indeed, the bud appears to form as the mesonephric duct brushes against that margin of the metanephric blastema (fig. 8). In only one nine day control had the bud failed to penetrate the metanephros and expand to form the typical needle-eye appearance.

The abnormalities of the developing excretory system of embryos of this age were largely limited to deviations of the mesonephric duct from the control pattern. These included interrupted growth, termination of the ducts anterior, adjacent, posterior, or lateral to the metanephric blastema. Failure of the ureteric bud to develop was noted in several instances; in some cases this was perhaps due to retarded development but in other specimens the absence was due to failure of the mesonephric duct to complete a normal course.

Nineteen embryos from four mothers were examined microscopically. From fourteen embryos with detectable abnormalities the following typical defects are described.

Only localized tubulations were seen for the mesonephric ducts of embryo 156G and K anterior to the metanephros. In embryo 156A the left and right ducts were markedly unequal, with regard to cross section along their lengths.

The left mesonephric duct of embryo 156A terminated just anterior to the metanephric condensation. In embryos 156D,E,I, and K, 163B, and 165A, the mesonephric ducts terminated at the metanephric blastema. In embryos 156D,K, 163B, and 165A, the ureteric buds did not develop on the sides affected. Even though the mesonephric duct reached the normal position adjacent to the cloaca, the ureteric buds did not form in embryo 125B.

A mesonephric duct passed laterally to the dermis in embryos 156A,F (fig. 9), 156G, 135A, and appeared to be directed in a similar course in a less mature embryo 156K. The right mesonephric duct of 156G (fig. 10) extended to the vicinity of the cloaca but then continued on to the adjacent dermis. A similar situation was seen in the development of the left mesonephric duct of 163A.

The right mesonephric condensation of embryo 156A was in contact with the cloaca but lacked a ureteric bud as the adjacent mesonephric duct passed toward the dermis.

A marked difference in extent of growth of the metanephric blastemata of embryo 156K was noted. The right blastema was 190 micra but the left only sixty micra in length.

At ten days, fourteen hours, control embryos have urogenital folds which have broadened considerably since the nine day stage. They are suspended by a medial band of tissue which is continuous with the mesenchyme lateral to the aorta (fig. 11). The width of this band increases as one progresses posteriorly to the caudal limit of the

metanephros. The ventral portion of the mesonephric folds are free and project medially toward the hindgut and subsequently merge with the mesenchyme dorsal to the cloaca. This junction is still closed by a mass of epithelial cells of both duct and cloacal origin. The Mullerian duct is dorsolateral to the mesonephric duct and keeps pace with the latter until the Wolffian duct begins to grow ventromedially toward the cloaca, at which point the Mullerian duct terminates.

The ureteric buds are seen rising medially from the mesonephric ducts very close to the developing urogenital sinus. The ureters pass craniodorsally to the metanephroi just anterior to the origin of the umbilical arteries. The pelvis of the kidney has expanded and subdivided into major and several minor calyces. Each of the calyces in turn branches into four or more subdivisions, each of which is capped by a dense aggregation of mesenchyme cells. The grouping of cells about the metanephros indicates the formation of the capsule. The urethra extends through a well-developed genital tubercle to open into the urogenital sinus.

The abnormalities of the developing urogenital system of embryos of this age included interruptions in the continuity of the mesonephric ducts anterior and posterior to the metanephric condensations, unusual relationships of the mesonephric ducts with the metanephric masses, unequal development of the right and left mesonephric ducts in the area posterior to the metanephric condensations, accessory ducts from the mesonephric ducts, and atypical orientation of the mesonephric ducts. (The term "accessory" is used throughout the report to designate any duct-like

derivative of the mesonephric duct which does not arise from the site which normally gives origin to the ureteric anlage.)

Fifty-six embryos from 10 mothers were examined. Thirty-eight were normal, 10 had developed abnormalities of the excretory system and 8 were recently dead. Nine of the embryos removed after 10 days, 1½ hours of development were compared with the remaining embryos which were sacrificed after 10 days, 14 hours development. No difference was noted between them other than extent of development.

The left urogenital ridge of embryo 159D was represented throughout most of its length by an irregular, dense association of cells. A short anterior tube was identified as part of the mesonephric duct. For some distance there was no duct or tubular structures to be seen. The duct reappeared for a short length only to terminate within 120 micra of the anterior pole of the left metanephric mass. Thirty micra posterior to the metanephric mass a duct progressed toward the cloaca and terminated there in the position ordinarily occupied by a mesonephric duct. There was no evidence suggesting the origin of this duct as either a mesonephric duct or a bud from the mesonephric duct. The right ureteric anlage had expanded and begun lobulation within the metanephric blastema of that side. No such development was seen on the left side.

The left mesonephric duct of embryo 159C was interrupted for 220 micra along its course but reappeared to continue to the point of origin of the normal ureteric bud. The bud entered the metanephric mass and elongated. The mesonephric duct terminated rather than continue to a position adjacent to the cloaca as did its mate.

Animal 146F developed a right mesonephric duct which passed lateral to mesonephric tubules bordering directly on the anterior pole of the metanephric condensation. The duct, however, continued into the mesenchyme of the posterior limb bud without sending off a ureteric bud or approaching the cloaca.

The mesonephric duct of embryo 159B passed to the integument anterior to the left metanephros and penetrated to the surface.

Subdivision of the pelvis had not been initiated in many of the ten day specimens but had advanced to a considerable extent in others. It is impossible to state how much of this variation was due to retardation subsequent to hypoxia as opposed to differences in ages of the litters based on fertilization time. In general, most members of a litter were in comparable stages of development.

Pelvic centers developed atypically in embryos 146G and 125B. In embryo 146G the mesonephric duct passed through the metanephric condensation. Orientation of the metanephric mesenchyme occurred about the mesonephric duct (fig. 12). In embryo 125B an accessory duct entered the cranial tip of the metanephric mass; the mesonephric duct successfully passed the caudal portion of the metanephric tissue but developed the usual ureteric bud which grew back to the metanephros and resulted in a second center for organization (fig. 13).

Posterior to the normal ureteric bud of embryo 176C, the right mesonephric duct passed into the limb mesenchyme rather than toward the cloaca.

At eleven days, fourteen hours, controls show further development of the Mullerian ducts as they parallel the mesonephric ducts. The urogenital fold is suspended dorsomedially by a narrow band of tissue. The gonads are densely cellular structures which are partly delimited from the mesonephros by a longitudinal groove. Progressing posteriorly in serial sections, one sees the supporting band of tissue broadening so that at the level of the metanephros the mesenchyme of the mesonephric ridge is continuous with that adjacent to the kidney blastema. Relationships of ducts at the urogenital sinus remain comparable to that seen at ten days as described above. Some of the more posterior mesonephric tubules may be patent now but do not show as extensive convolutions as the anterior tubules and do not open into the mesonephric duct. The metanephric kidneys are lateral to the aorta and the right kidney is further separated from the aorta by the post cava which is developing by an anastomosis of right subcardinal and postcardinal veins.. Development of the nephrons has progressed to the formation of S-shaped units seen in close association with an increasingly sub-divided system of collecting ducts. The potential renal arteries may be seen as buds adjacent to the metanephroi (fig. 14).

Twenty-nine embryos obtained from 6 mothers were studied microscopically. Eleven of these were sacrificed at 11 days, 1½ hours, the remaining 18 were fixed at 11 days, 14 hours. Of the 29, 3 were dead, 9 normal, and 17 presented abnormalities of the developing excretory system.

Abnormalities of the embryos sacrificed on the 11th day of gestation were quite varied. Five embryos, 177B, 137C,B,C, and G, showed bilateral renal agenesis. Four other specimens developed only one kidney. In two embryos the metanephroi developed ventral rather than lateral to the aorta and were separated only by a capillary or a few cells so that one would anticipate a fusion of the kidneys in 166C and 172C (fig. 15). Embryo 166F showed metanephroi ventral to the aorta but if fusion were to occur in this case it would have been fusion between the head of one kidney and the caudal tip of the other (fig. 16).

Bilateral agenesis in 177B was associated with the termination of the mesonephric ducts before formation of the ureters. In this instance the left mesonephric duct terminated at the point where the urogenital fold fused laterally with the peritoneum. The right mesonephric duct terminated at approximately the level where a dorsally directed diverticulum could be seen passing from the duct toward the pelvic musculature. In embryo 137A, agenesis was associated with divergence of the left mesonephric duct toward the dermis and early termination of the right mesonephric duct before reaching the position at which it ordinarily would give rise to a ureteric bud (figs. 17, 18). The third case of bilateral agenesis occurred in the same litter. The left mesonephric duct passed from the urogenital ridge into the tissue close to the aorta and terminated there. The mesonephric duct of the opposite side appeared normal but if a ureter formed from it, the latter failed to reach the metanephric condensation. Sections within which the mesonephric duct of that side terminated were lost during processing. In embryo 137C the left mesonephric

duct passed to the dermis and the right ureter failed to reach the metanephric mass and terminated with the umbilical artery between it and the metanephric blastema. In embryo 137G both ridges fused with the peritoneum (fig. 19) and both mesonephric ducts passed lateral to the umbilical arteries. The left mesonephric duct, after passing dorsomedially, penetrated to the epithelium of the skin (fig. 20); the right duct terminated in the developing pelvic musculature.

Unilateral renal agenesis was associated with the passage of the mesonephric duct to the dermis in 137D, and inadequate development of the urogenital ridge in 177C and 177B.

Abnormalities of the excretory ducts for the metanephros were seen in several embryos. In embryo 172D the right mesonephric duct developed an accessory bud which passed to the metanephric condensation and the combination produced a normal kidney. The mesonephric duct did not develop, however, beyond the point of origin of the accessory duct so that there was no provision for removal of wastes from the kidney. The kidney of the opposite side also received an accessory duct from the mesonephric duct (fig. 21). The mesonephric duct itself passed next to the metanephric mass and about it (or possibly a second accessory duct at this point) further orientation of the metanephric condensation occurred (fig. 22). The pelvis developing about accessory duct number 1 and about the mesonephric duct slightly posterior to it were continuous with each other. Finally a ureter developed from the customary site of this mesonephric duct and assumed its normal relationship with the metanephric material remaining and developed an independent association of

of mesenchyme cells about its terminal expansions (fig. 23). Thus this kidney was supplied with three efferent ducts which utilized two main ducts for progression to the mesonephric duct-ureter junction by the urogenital sinus. Two independent pelves had developed.

An accessory duct served as the basis for development of the cranial part of the right kidney of 172E but in this instance the mesonephric duct continued to the vicinity of the urogenital sinus and gave rise to a ureteric bud about which the remainder of the metanephric material became organized. (This is somewhat comparable to the condition described for the left kidney of 172D).

In embryo 177C an inadequate right and left urogenital ridge development was responsible for termination of both mesonephric ducts approximately 600 micra anterior to the last trace of the ridge. The right kidney did not develop beyond the condensation stage. A mesonephric-like duct appeared lateral to the right umbilical artery and some 60 micra posterior to the last vestiges of the urogenital ridge (thus some 660 micra posterior to the last traces of the mesonephric duct). This terminated on the right side of the umbilical artery without making contact with the left metanephros. Recall that the left mesonephric duct had also terminated toward the end of the urogenital ridge. A ureter connected a developing left metanephros (fig. 24) with tissue immediately adjacent to the urogenital sinus on the left side (fig. 25). The position with regard to both kidney and urogenital sinus was normal. The kidney was seen through 43 sections of 10 micra in thickness. There was no evidence of this ureter developing as a lateral bud from any other

structure in the sinus area, as is the usual case, or arising from a remnant of a more lateral mesonephric duct.

At twelve days, fourteen hours, controls are characterized by a considerable development of the nephron and collecting duct system of the metanephros (fig. 26). The mesonephric fold is supported dorsally by only a thin strand of tissue. The gonads are suspended by an equally thin cord of tissue from the mesonephric fold. The mesonephric folds swing lateral to the gonads and within this broadest portion the Mullerian and mesonephric ducts arch ventromedially. Posterior to the gonads the mesonephric folds are supported more broadly along their medial margins as they pass the more caudal portion of the metanephroi. The Mullerian ducts, which originate laterad of the mesonephric ducts, sweep ventromedially of the latter to approach each other. The mesonephric ducts follow essentially the same path but drop sharply, as the Mullerian ducts approach one another, to join the bladder in conjunction with the ureters (fig. 27). It is difficult to determine whether the ureters actually open into the bladder directly or depend on the termination of the mesonephric ducts which have finally opened into the bladder.

Tubules are still seen in the mesonephric fold. None of these make contact with the mesonephric duct. The more posterior tubules are now the most outstanding but even at this peak of their development there is not the same intimate association with the mesonephric duct shown by the anterior tubules at an earlier stage of development.

Fourteen embryos were obtained from four mothers after 12 days and 14 hours of development. Four of the fourteen embryos revealed abnormalities

of the excretory system.

Abnormalities of the developing excretory system of embryo sacrificed at this age were generally comparable to those described in the 11 day group. Three embryos were found to have unilateral renal agenesis. One embryo had a hypoplastic kidney. Accessory ducts were noted in some instances. Abnormal locations of mesonephric ducts were noted among this group.

The right urogenital ridges in embryos 131E and 142B were abnormally curtailed in length. In both embryos mesonephric ducts failed to develop adequately to produce ureteric buds. Unilateral agenesis occurred in both instances. The urogenital ridge on the left side of embryo 131A fused posteriorly with the lateral peritoneum and served as a bridge for a lateral excursion of the mesonephric duct which passed to the dermis and terminated. Left renal agenesis occurred in this specimen (fig. 28).

A small kidney developed about the terminal expansions of an accessory duct which developed along the mesonephric duct of embryo 131C. The point of origin of the accessory duct (fig. 29) was such that the "ureter-like" structure passed dorsomedially to reach the metanephric condensation (fig. 30). Under these circumstances it would be expected that any urine which would form would pass through the "ureter", into the mesonephric duct and down into the bladder if the mesonephric duct persisted and opened into the bladder. One hundred and ninety micra posterior to the origin of the accessory duct a second accessory duct developed from the mesonephric duct and passed dorsally toward the vertebral column into an area which would be continuous posteriorly with the metanephric area. There

was no condensation of cells or development of tubules in the vicinity of the end of this duct (fig. 31). The mesonephric duct terminated at the point of origin of the second accessory duct so there remained no provisions for removal of wastes from the kidney.

Two large-size ducts developed medially from the mesonephric duct of embryo 142B. These ducts served to connect the mesonephric tubules which were developing just posterior to the metanephric kidney (fig. 32).

In embryos 131C,D, and embryo 142B, well developed clusters of mesonephric tubules developed either medial, ventral, or just posterior to the developing metanephros. Only in the case of 142B, described above, however, was there any provision for drainage of these structures. In embryo 131A the tubules developed in the location ordinarily occupied by the metanephros.

In embryo 131A the Mullerian duct, ureter, and mesonephric ducts merged and terminated before approaching the bladder. This animal also had unilateral renal agenesis.

Table 5 includes all abnormalities of the developing excretory systems of the embryos which were studied microscopically with one exception. The mesonephric duct of a ten day embryo passed directly through the metanephric blastema. This particular defect did not fit into any of the major categories included in the table. The figures in tables 4 and 5 do not afford an opportunity to evaluate any tendency for more frequent abnormal development of the left or right side. One reason for this is that if a ureter fails to develop from an otherwise normal mesonephric

TABLE 5

ABNORMALITIES OF THE DEVELOPING EXCRETORY SYSTEM DETECTED IN MICROSCOPIC EXAMINATION OF 9, 10, 11 AND 12 DAY EMBRYOS OF MOTHERS EXPOSED TO 220 MM. HG FOR TEN HOURS FROM 1:30 A.M. TO 11:30 A.M. ON THE EIGHTH DAY OF GESTATION

Age at time of sacrifice	Abnormal animals	Ridge development	Mesonephric duct discontinuous anterior to metanephros	Termination anterior to metanephros	Termination adjacent to metanephros	Termination lateral to metanephros	Termination posterior to metanephros	Accessory ducts to metanephros	Accessory ducts to non-metanephric areas	No ureteric bud	Abnormal ureteric bud growth	Multiple pelaves	Abnormal metanephric position	Hypoplasia	Agenesis	No throughway from metanephros to exterior
9 days 14 hrs.	14	-	3	2	7	5	1	-	2	-	-	-	1	1	-	-
10 days 1.5-14 hrs.	10	-	4	0	0	3	1	2	0	4	-	2	0	0	-	-
11 days 1.5-14 hrs.	17	10	6	3	1	6	2	3	1	14	1	2	6	0	15	1
12 days 1.5-14 hrs.	4	3	0	1	3	1	0	2	2	3	0	0	0	1	3	2
Totals	45	13	13	6	11	15	4	6	5	21	1	4	7	2	18	3

duct, two abnormalities have been recorded for that side although on the basis of our present knowledge, one of these is the direct cause of the other. By limiting as many known or most probable instances of cause and effect to a single point value, an approximation of one sidedness can be made. From the figures of the abnormalities detected microscopically, a ratio of left to right sided defects of 53 : 27 was obtained. The gross dissections yielded a ration of 32 left to 26 right but it must be remembered that the information available for these specimens is not as great as that obtained from the embryos studied microscopically. Combining the results of the two categories, a ration of 85 left to 53 right is obtained.

In the course of gross or histologic examination of 193 embryos between 11 and 16 days of age derived from the uteri of 32 females which had been exposed to hypoxia on the 8th day of gestation, 7 embryos were characterized by bilateral and 25 by unilateral renal agenesis. Hypoplastic kidneys were seen in 5 embryos. The metanephroi were malpositioned in 10 specimens. Because sex determination could not be made on the majority of microscopic specimens, it is not possible to give sex ratios for abnormalities of the excretory system except for the 15-16 day embryos which were dissected and examined grossly.

#### Other Defects

In addition to those described for the excretory system, there were abnormalities of skeletal, nervous, digestive and respiratory systems. The skeletal systems of a number of full term embryos which had survived various periods of hypoxia during the 7th through the 10th day of gestation

showed axial defects. By using the alizarin technique to stain the bone, it was possible to see that the more anterior defects were found in embryos exposed to hypoxia in the earlier stages of development and that the older the embryo at the time of hypoxic insult, the more caudad were the vertebral anomalies. The defects included micrognathy, anencephaly, fused and absent ribs, a greater frequency of supernumerary ribs, anomalous formation of vertebrae from the thoracic to the sacral area, and retardation of limb formation from absence of one digit to complete failure of limb development (fig. 33).

Hare-lip, both uni- and bilateral, was observed (fig. 33). Herniated viscera were seen only in experimental animals (figs. 33, 34). The only anomaly detected in the controls was protrusion of the brain through the top of the cranium. It occurred in a single animal, but was found also in a small number of the experimental group.

Seventy-six of one-hundred fourteen embryos had defects of the spinal cord (figs. 17, 20, 35). The abnormalities were located predominantly in the more anterior half of the cord but were also found in the posterior half and occasionally were limited to the posterior portion. Thirteen of the 30 embryos sacrificed after 11-12 days of development had hernias of the diaphragm with hepatic tissue extending along the length of the pleural cavities for as much as 400 micra (fig. 36). The liver masses were continuous with the main body of the liver by tenous cords of parenchyma. Observations of abnormalities of the reproductive system, aside from those which would result from failures associated with the mesonephros as detected in the microscopic study, were limited to

occasional reduction in size of the developing gonad in instances in which the genital ridge failed to develop to full size. In the dissected full term embryos the abnormalities of the reproductive system included hypoplasia of the ovary and absence of the uterine horn. In the male the testes were occasionally displaced and in one specimen two left testes were identified histologically. One of these was just posterior to the normal kidney area and the other had descended to its normal position (fig. 37).

## DISCUSSION

### Timing

The preliminary exposure of gravid hamsters to a single five hour period of hypoxia on the 7th, 8th, 9th, or 10th day reveals that for this animal, as has been described for the mouse (Ingalls, et al, 1952) and rabbit (Degenhardt, 1954), there is a period of susceptibility of the embryo to maternal hypoxia (as well as to other acutely acting teratogens) which coincides with the period of rapid morphogenetic differentiation (neural tube formation, formation of somites, posterior development of the mesonephric duct) and with the proposed chemical differentiation of anlagen which have not yet differentiated morphologically. In the rabbit, a sensitivity was noted during the period from the 8th to the 10th day in a gestation period of 37 days. In the mouse, the sensitive period lies during the 7½ to 12½ day interval of a gestation period of 19 to 20 days. In the hamster, a marked sensitivity to hypoxia occurs during the period from 7 to 9 days (postovulatory) of a gestation period of 15½ to 16 days. During the short span of time from day 7 to 8, the yolk sac blood islands begin to coalesce and the heart begins to form, the primitive streak elongates, the medullary plate thickens and the head process develops. The neural ridges which were elevated on the 7th day meet dorsally over the first 6 somites as the latter appear late in the 7th day. In the early 8th day the tail undergoes a slight torsion as it flexes ventrally to bring its tip to a position adjacent to the right side of the head. During this time the 7th through the 23rd somites develop. By the 9th

day the flexion is not as pronounced, the body is in one plane, and the anlagen of all major organs are well defined. It was during the period of most rapid change in body form and somite proliferation that the embryos were subjected to the environmental alteration.

### Congenital Anomalies

#### Excretory system

The renal agenesis noted in preliminary studies was investigated histologically to determine the first morphological indication of impending abnormalities of the excretory system. The mesonephric duct is in the process of formation in the anterior somites and the posterior somites are forming during the 8th day of development. Examination of embryos sacrificed on the 8th day failed to reveal any defects of the nephrogenic cord. The number of intersomitic clefts which had developed in test embryos was often less than in the controls and the conversion of the nephrogenic cord into medial and lateral ridges within which the tubules and duct respectively would develop was similarly retarded. A "ragged" appearance of peripheral cells was noted in the somites of some embryos.

Posterior progression of the mesonephric duct and tubule formation.

The 9th day embryo offered a more favorable opportunity for comparison of development of the mesonephric ducts and tubules than the earlier specimen. It was at this age that the first definite irregularities in development could be detected. The discontinuity of the mesonephric ducts observed in two nine day specimens is far more noticeable in 10th and 11th day embryos. It would be necessary to take a large number of closely timed specimens to determine if the interruption described

occurred after the normal duct had formed completely and was the result of degeneration or if the portions of the duct arose without reference to a terminal bud.

Let us assume that the raising of the nephrogenic ridge, as seen in the late 8 day embryo, is evidence of successful induction of the cord to form a mesonephric duct. If at any point there is a metabolic inadequacy or inducer or of cells, that part of the cord will fail to differentiate into duct. This would be of no consequence for, according to the theory, the duct could develop successfully posterior to the break. That this theory is inadequate, however, is evident when one looks for and fails to find mesonephric duct posterior to the point where apparently healthy, but aberrant mesonephric ducts depart from their normal course and pass, for example, to the skin. An additional difficulty with the theory is that one might expect cellular competence for differentiation into mesonephric duct to be limited to the nephrogenic ridge. This certainly is not the case.

The alternative theory, that development of the mesonephric duct occurs by progression of a stimulus - possibly a growing point - from the anterior end of the nephrogenic cord to the cloaca is more compatible with the findings of Boyden (1927), Grunwald (1937), Waddington (1938), Miura (1930), and Holtfreter (1944). If it can be assumed that the nephric cord is responsible for contact guidance of "selective conduction" (Weiss, 1947) of the growing tip of the mesonephric duct toward the cloaca, one would anticipate an alteration in normal pattern of Wolfian duct development to occur wherever there is an interruption in the guidance mechanism of the substrate. It has been noted that interpretation

of the status of the nephric cord in the 9 day embryos is rather difficult. By the tenth day, however, the deficiencies of the duct are exaggerated for at this time there are lengths of 200 to 400 micra of ridge in which there is no evidence of mesonephric duct or of necrotic tissue. Mesonephric tubules fail to develop in those parts of the ridge from which the duct is absent. It will be recalled that when Gruenwald (1942) introduced non-nephrogenic mesenchyme into the path of an elongating Wolffian duct, the duct either terminated abruptly, turned to the side, passed through the obstruction and proceeded to the cloaca, or, in two instances, was diverted by the obstacle and grew laterally to the ectoderm. A metabolic disturbance in a localized portion of the rapidly dividing somites during the ten hour exposure to hypoxia could conceivably result in the establishment of a molecular pattern foreign to the guidance system ordinarily responsible for the orderly progression of the mesonephric duct to the cloaca. Such a knot of tissue might be incompatible with the growing tip of the Wolffian duct and either inhibit its caudal elongation, cause it to be diverted from its path, or alter the metabolism of the growing tip so that further differentiation and elongation is no longer possible. Willier (1954) observed that during the phase of organogenesis "a localized disturbance in a developing primordium, or its precursor material, may disrupt its subsequent development, and as a consequence it develops abnormally." The diversion of mesonephric ducts noted in several instances might be a reflection of such a local phenomenon. The absence of mesonephric duct for the indicated lengths could be due to localized trauma to the cells of the developing duct which would prevent tubulation and differentiation but without

causing cell death and appearance of necrotic tissue. A normal complementary differentiation of ridge tissue into mesonephric tubules, dependent on the local integrity of the mesonephric duct, would be a secondary effect and thus account for the experimental results described above.

#### Development of the urogenital ridge

The ten-day embryo offers the first chance to evaluate the overall structure of the urogenital ridge. In both ten and eleven day experimental animals the urogenital ridge was seen to undergo abnormal development. In the controls the ridge was suspended within the body cavity by a dorsal band of tissue with the coelom separating the ridge laterally from the parietal peritoneum (fig. 11). The abnormality of the ridge in the specimens mentioned consists of a continuity of the ridge with the parietal peritoneum either by formation of a mid-ventral cleft in the ridge with the lateral leaf merging with the body wall (fig. 17,18) or by failure of the ridge to project into coelom and become free of the abdominal wall (fig. 19). One other example of anomalous development of the ridge was simple hypoplasia in which case the ridge terminated by tapering down to insignificance either anterior or posterior to the genital anlage.

In the instance of atypical relationship of the urogenital ridge with the body wall, the mesonephric duct had an alternative to remain within the ridge or to pass laterally to the body wall. It has been noted that the latter course was followed in a number of instances as illustrated by the path of the duct in figure 20. In the more posterior

portions of the body cavity the urogenital ridge does fuse with the ventrolateral peritoneum but there was only one instance of a mesonephric duct developing abnormally in this area.

The successful progression of the mesonephric duct through the differentiating mesenchyme of the body wall serves notice that development of the mesonephric duct does not depend upon accretion of cells from within the urogenital ridge.

#### Metanephric development

Metanephric development did not occur in any instance in which there was failure of a duct to penetrate the blastema. This finding is in agreement with the evidence of a normal inductor relationship between the ureteric bud and metanephric condensation noted by Schreiner (1902), Pohlman (1905), Fischel (1912), Boyden (1927), Brown (1931) Grunwald (1937), Gruenwald (1942), Waddington (1936), Gluecksohn Schoenheimer (1943), and others.

The metanephros in the experimental hamster, however, did develop with reference to ducts other than the ureter. A combination of all individual capacities seen in the various embryos in this experiment has been found in embryo 172D. In this specimen pelves develop from the normal ureter (fig. 23), from an expansion of the mesonephric duct itself as it passed through a portion of the condensation (fig. 22), and about the termination of a more anterior accessory duct derived from the mesonephric duct (fig. 21). The capacity to induce organization of metanephric cells is thus a property of the mesonephric duct, the normal growing tip of the ureter, and accessory ducts. It appears that the ureter and accessory ducts may well be homologues of mesonephric collecting ducts that

have been described in the amphibian and the chick. The underlying mechanism which stimulates formation and directs the projection of the ureteric bud to the metanephros is not known but may ordinarily be a guidance system such as that proposed for the posterior progression of the mesonephric duct.

This in vivo evidence of a more wide spread inductive capacity than is normally exerted is of interest in regard to Grobstein's (1957) in vitro demonstration of the inductive capacity of mouse spinal cord to cause differentiation of kidney tubules of mouse metanephrogenic mesenchyme. The induction of nephric structures by neural tissue was originally suggested by Gruenwald (1942) who noted the mesonephric tubules developing in association with a ganglion in the absence of the mesonephric duct. Auerbach and Grobstein (1958) demonstrated that the epithelial bud of the mouse submandibular gland also possesses in vitro inductive capacity toward the mouse metanephrogenic mesenchyme.

Double ureters have been described in experimental animals and in pathology, but both ureters ordinarily open into the bladder, or, in some instances, ectopically into lower parts of the urogenital passages. The only instance of double ureter in this experiment resulted from the combination of a normal ureter and an abnormally located mesonephric duct described above. It is not known what the fate of the mesonephric ureter and its nephrons would have been had the embryo gone to term.

That accessory ducts are not in all instances normal ureteric buds developing from mesonephric ducts is also proved by embryo 172D

described above. There does remain the possibility, however, that the mesonephric ducts have produced normal ureteric buds in their progression toward the cloaca but ceased elongating shortly after bud formation and thus failed to carry the base of the ureter along to the cloacal area. Such a possibility is indicated in some of the nine day embryos. This then would equate ureters and "accessory ducts" in some instances, the only distinction in such cases being either the direction or the point from which the two approach the metanephros.

I have been unable to find a reference to the utilization of the ureter as a common duct for both excretory and reproductive products. Such a circumstance can be anticipated in examination of embryo 36C in which the gross dissection shows the left testis held close to a ureter which presumably is actually a mesonephric duct. In this instance the duct continues to the bladder. In embryo 38J the duct which leaves the pelvis of the kidney extends to an undescended testis and terminates. A similar relationship and origin exists here.

Among the experimental animals were some in which the metanephric condensation underwent normal development after induction by accessory ducts which would afford no outlet for the excretory products. The resulting development would be a cystic kidney but of a type which I have not found described in texts of human embryology or pathology.

#### Hypoplasia of the metanephros

The metanephrogenic condensation was present in all embryos that were examined histologically. A change was apparent in the tissue if induction had not occurred before the 12th day. A rather granular appearance

developed in the mass and the staining characteristics were less intense.

In two instances the condensations were reduced in size. No explanation is offered for the one-third normal size metanephric mass observed in a nine day embryo except that this may be an instance of a latent expression of hypoxia, for there is no evidence of a metanephric mass during the 8th day. The hypoplasia observed in the 11th day embryo was associated with induction by an accessory duct. Gluecksohn-Schoenheimer (1949) observed that whatever kidney develops in mutant strain Sd+ mice, there is always a normal quantitative relationship of medulla and cortex. On the basis of her observation and the devious method of induction in this metanephros, it seems quite likely that in both instances either the inducing structure is not equivalent to the normal physiological inducer or the inducer has arrived tardily at its destination and some of the waiting tissue has lost its competence. Whatever inducer is present would be capable of causing differentiation of metanephrogenic tubules only to an extent comparable to its own ability to form a system of collecting ducts.

#### Abnormal position of the metanephric kidney

The failure of the metanephric masses to ascend to their normal lateral positions is assigned to regional abnormalities rather than to a deficiency of the metanephric tissue itself. The development of collecting units within the metanephric mass was either normal or had differentiated to an extent comparable to embryos of similar age after exposure to maternal hypoxia. The umbilical arteries were in a position to

interfere with normal migration. This, in some respects, is reminiscent of anomalies of the iliac plexus observed by Brown (1931) in mice and said to account for abnormal pressure or distortion of the tissues in the lumbosacral area. The possible role of the umbilical arteries will be considered in the section on vascular relationships.

The abnormal development of the metanephroi in which their final position was immediately adjacent to one another so as to produce adherence, if not actual fusion, has been noted in other teratologic studies, including that of Wilson and Warkany (1948) who studied malformations in the genito-urinary tract in the embryos of vitamin A deficient rats. In their investigations there was no evidence of umbilical arteries exerting undue pressure on the migrating kidneys. Sikov and Noonan (1958) observed similar abnormalities in rats from mothers to which radiophosphorus had been administered. The basis of the abnormal position in their study was not indicated.

The relationship of urogenital abnormalities to neural and skeletal defects.

Because of the significance of the mesoderm and neural tube in organizing functions of vertebrates, a survey has been made of the relationship of urogenital abnormalities to neural and skeletal defects. Gluecksohn-Schoenheimer (1949) and Watterson (1954) have shown that in mice and chicks, respectively, the qualitative and quantitative formation of cartilage of the neural arches is dependent on the amount of neural tube present. It has been shown that the formation of cartilage which ultimately undergoes endochondral ossification in formation of the centrum

of vertebrae is dependent upon the presence of the notochord in the chick.

Eighty-three percent of 114 embryos from females exposed to hypoxia for 5 hours during the 7th or 8th day suffered abnormal development of the axial skeleton. Sixty-seven percent of the embryos examined histologically (the 8 day 10 hour series) had abnormalities of the spinal cord. Thirty-eight of the 45 embryos with abnormalities of the excretory system also had spinal defects. There were seven embryos which had no spinal defects associated with abnormalities of the excretory system. In view of the overall high percentage of skeletal defects and the frequency of spinal abnormalities among the embryos, it is quite possible that the relationship between abnormalities of the three systems is due to more than coincidental sensitivity to hypoxia. The study of more fully developed embryos than those examined histologically in this work would shed light upon this problem. Smith (1958) determined the dependency of the sclerotome of the hamster upon neural induction and commented as follows:

The inductor principle of the neural tube which yields sclerotome in the formation of the vertebrae (demonstrated by Grobstein in the mouse) can be demonstrated in the hamster. As explantations are made on successively later days in development the percentage of the anlagen which develop into recognizable vertebral elements rises sharply. These indicate that the inductor systems have, by 8.5 days of development, irreversibly determined the fate of their respective mesoderms. This is true of both the limb bud inductor system and that of the neural tube.

If the role of this inductor system could be extended to the nephrogenic ridge it would be of significance in explaining the confinement of excretory abnormalities observed to embryos of mothers which had been exposed to hypoxia during the first ten hours of the 8th day of gestation.

Gruneberg (1952) states that "the development of the uro-rectal syndrome as the result of many different genetic situations [in mice] has been used as evidence for the existence of an inductive relationship connecting the axial skeleton with the hindgut and urogenital system." There was no evidence of abnormality of the notochord associated with skeletal or neural defects in the histological preparations of the present work.

#### Vascular relationships

Failure of the mesonephric duct to approach the metanephric condensation in embryo 156A may have been related to the presence of an irregular, small vessel which lay between the metanephros and the duct. This would not seem, however, to account for the failure of the duct to continue to the cloaca rather than passing laterally to the adjacent mesenchyme.

The relationship of the post- and sub-cardinal veins in the hamster is not comparable to the situation seen in animals such as the pig, where the mesonephros attains a functional capacity. The sub-cardinals are large and from them develop the glomeruli associated with the mesonephric tubules. In the hamster, the subcardinals are in the vicinity of the mesonephros but consist mainly of small anastomosing passageways at times closely associated with the tubules, but at other times not visible for a number of sections. Their development is significant only in the area where they combine with the posterior cardinals and the caval fold in the development of the post cava vein in the vicinity of the metanephroi. Glomeruli have not been observed in association with the mesonephros of the hamster.

According to Boyer (1948) the left umbilical artery undergoes regression beginning on day 12. A variation from the normal pattern occurred in the absence of the left umbilical artery in 5 animals and of the right in one other. The abnormal medial position of the kidneys in embryos 166C,D, and 172G was possibly determined by the interrelationship of the umbilical arteries and the urogenital ridge for in these specimens the kidneys appeared to be wedged between the arteries ventral to the origin of the latter from the dorsal aorta (fig. 16).

Brown (1931) observed that the frequency of excretory abnormalities in the descendants of x-rayed mice was due to aberrant vascular patterns. She described inadequacies of blood vessels such as agenesis, hypoplasia, distortion, and rupture of vessels of the iliac plexus as being most significant. Agenesis in her mice was due largely to failure of the normally located ureter to pass through the aberrant vascular plexus to the metanephros. Agenesis in the present work was largely due to the failure of the mesonephric ducts to approach the cloaca with ureters "in hand". Brown believed abnormal position of the kidneys was determined largely by the structure and adequacy of the umbilical arteries at the time of body torsion. That the same time factor is significant in the experimental hamster has already been suggested. I am not prepared, however, to indict the umbilical arteries in the same way.

Distribution of abnormalities of the excretory system.

Little can be said about the greater frequency of abnormalities noted for the left as opposed to the right side of the urogenital system.

Herniation of the diaphragm in these animals displayed a pure right-handedness. It is a fact that certain abnormalities have a predilection for one or the other side reminiscent of the tendencies of individuals toward right versus left handedness. Landauer (1939) studied the frequency of supernumerary nipples and other abnormalities. In the instance mentioned it was found that 45.8% of the cases of hyperthelism were on the right, and 54.2% on the left side of the body. Furthermore, there was greater frequency of the abnormality in men than in women. Carpenter and Potter (1959) report a 3:1 ratio of males to females in frequency of bilateral renal agenesis in the human. Gluecksohn-Schoenheimer (1943) observed a left hand tendency in occurrence of urogenital abnormalities in the Sd strain of mutant mice.

Bagg (1925) observed a ratio of 140:145 cases of right to left renal agenesis. Brown (1931, working on the Bagg line, noted that "the failure in development of the right kidney is twenty percent more frequent than the left kidney in 9-12 day stage of development. But from this time on to term, probably through absorption and death of defective embryos, the number of kidney failures is found to be the same on the two sides." Brown believed that the direction of twisting of the body in early stages of development is related to the stage of development of the umbilical arteries. If there is a twisting to the left, the left metanephros is more subject to pressure while the right side is comparatively stretched. Her results indicated that the compression was more detrimental than stretching and she held this to be an additional factor in determining which side would be more susceptible to

irregularities of excretory development.

In view of the absence of any apparent causative factor involved in the left-handed tendency noted in abnormalities of the excretory system in this study, the condition will simply have to pass recorded as an observation.

#### Other abnormalities

It has been observed that a wide variety of abnormal conditions occurred in both gross and histological examination of the embryos in this study. It is interesting to observe that all teratogens, when applied during the most susceptible periods of embryonic development, generally result in a broad array of defects. Often the systems involved are identical; not infrequently the same structures are affected, sometimes in quite similar fashion, other times in different ways. The spectrum of abnormalities associated with any one teratogen acting in a given period on a selected organism is reminiscent of the phenomenon of pleiotropism in genetics in which one gene is associated with the production of a similarly large number of abnormalities. As an illustration of this situation four lists are presented in table 6. The work on the rat has been done by Wilson (1953), on human material by Carpentier and Potter (1959), and Potter (1946), and on the mouse by Hummel (1959).

Hummel (1959) described a semi-dominant lethal gene Disorganization (DS) which "differs from other deleterious genes of the mouse in several respects; the variety of expression or pleiotropism in heterozygotes, the wide distribution of abnormalities, their random distribution;

the lack of patterning of multiple defects; and certain of the types of anomalies." She adds that "disorganization appears to be active and effective as a teratogen during the entire period of major organogenesis in contrast to other genes and extrinsic teratogens which act for shorter periods on specific developmental stages. ... Disorganization appears to disturb some basic metabolic process thereby causing overgrowth, misplacement, and general upset in inductive relationships."

TABLE 6

ITEMIZATION OF PLEIOTROPIC EFFECT IN TERATOLOGY, ASSOCIATED ABNORMALITIES IN HUMAN AUTOPTSY MATERIAL IN CASES OF BILATERAL RENAL AGENESIS, AND TRUE HEREDITARY PLEIOTROPISM IN MICE

Hamster	Rat	Human	Mouse
Hypoxia	Vitamin A Deficiency	Autopsy	DS Mutation
exencephaly micrognathia hare-lip vertebral and rib anomalies limb defects cryptorchidism renal agenesis hydroureter uterine aplasia herniated diaphragm abnormal spinal cord	eye abnormalities aortic arch defects lung anomalies heart defects diaphragmatic hernia renal abnormalities ectopic ureters abnormal genital ducts	anencephaly abdominal testes septal defects ano-rectal malformations limb abnormalities hypoplastic limbs renal agenesis uterine aplasia	cranioschisis exencephaly hamartomas anomalies of appendages eye defects gastroschisis defects of tail anomalies of digestive tract urogenital defects diaphragmatic hernia

The Hypoxic Mechanism Causing  
Congenital Abnormalities

There is no unifying principle which can be applied to account for all the abnormalities identified. The structures affected are of ectodermal origin in some instances and of mesodermal origin in others. Some were involved in tubulation, others in formation of supporting structures; some were destined to become epithelial units while others would function as connective tissue or neural elements. The only basis for comparison lay in their common involvement in the processes of cellular movement, growth, division, differentiation, or surface-related phenomena including migration of cells to appropriate positions and their association with inductive processes. All these factors are playing within each individual genetic background.

The effect may well have been related to (1) retardation or other interference in development by decrease in the amount of oxygen available, (2) accumulation of toxic substances or (3) interruption of normal placental transfer including possible contamination with maternal substances through abnormal permeability of placental structures.

Embryos are known to be able to function under anaerobic conditions for some periods of time. E.J.Boell (1955) states that:

A comparative study of the rate of anaerobic glycolysis and respiration in presomite and early somite rat embryos has revealed the interesting fact that the embryo can derive more energy from the anaerobic breakdown of glucose than from oxidation [Boell and Nicholas, unpublished]. This may have considerable significance for the embryo, for a condition of "Everest in utero" probably exists during the period before placentation as well as at birth.

It has been noted by teratologists that the rat is resistant to congenital

defects on exposure to hypoxic conditions.

Other factors which may be involved in the response of the embryos to hypoxia would include the degree of oxygen saturation of the blood, and the response of the embryonic system to a decrease in oxygen tension. Prysnowsky (1957) studied the placental structure of mammals and its relationship to oxygen pressure gradient between maternal and fetal bloods. He observed that "the oxygen capacity of embryonic blood is higher than that of maternal, but its [embryonic] saturation is lower than that of maternal blood." Dawes (1955) found that the fetal lamb also has a low percentage of oxygen saturation of the blood. A study of fetal blood pressure and umbilical flow revealed that "the sympathetic system of the lamb is adequate to give typical adult responses of increased blood pressure, heart rate, umbilical flow and oxygen capacity of blood (largely through contraction of the spleen)." This latter consideration, however, would not enter into the problem of hypoxia in the 8 day hamster embryo.

An additional factor which might be involved in determining the relative sensitivity of hamster embryos to hypoxia in comparison to those of other species might be found in physiological mechanisms which assist the maternal organism of a hibernator to adjust to lowered oxygen tension. I have no suggestion as to the possible significance of this factor on either maternal survival or embryonic morbidity or mortality. In a study of the comparative resistance of hibernators and non-hibernators, Hiestand, Rockhold, Stemler, Stullken and Wiebers (1950) observed that "the greater hypoxic resistance of the young appears to be due to such factors as incomplete homeothermism, cerebral metabolic rate, and glycolytic ability. ... It is suggested that good anoxic resistance is a

prerequisite to ability to hibernate."

#### Genetic Aspects of The Problem

The animals used in these experiments were non pedigreed albino hamsters in which no excretory abnormalities had been detected. The question might be asked if the frequency of any teratogen-induced abnormality might not be assigned to the enhanced susceptibility of a certain genetic composition. Landauer (1954) noted a facial defect rate of  $60.3 \pm 3.42$  for White Leghorns and  $93.7 \pm 1.57$  for Silver Gray Dorkings which had been exposed to the same 2.5 mg. boric acid teratogen after 96 hours of incubation. Just as there are modifiers and factors for incomplete penetrance in genetic studies, so one would anticipate that these factors would also modify the results obtained in a study of teratogen induced abnormalities as proven by Landauer. While an experiment using non in-bred animals does not permit close comparison of figures with those obtained from a genetically controlled experiment, it is quite possible that the variety of effects detected in this study exceeds those which would be identified under other experimental conditions. In studying inductor relationships it may be advantageous to obtain as much variation as possible.

The lists of congenital abnormalities found at autopsy, from teratology, and from hereditary factors are most interesting in the similarity of structures affected, often times in association with urogenital abnormalities. The only references found on urogenital abnormalities in the hamster which are based on genetic effect are those reported by Magalhaes (1959) and Orsini (1952). Magalhaes described renal agenesis, cystic

kidneys, abnormal uteri, and other abnormalities in a strain of white-spotted golden hamsters. Orsini observed aplasia in the female urogenital tract of a group of piebald hamsters. Neither report indicated that the genetics of the abnormalities had been worked out. Since the present work was done on albino hamsters, it would be of interest to see if there is any relationship of coat color and tendency to certain urogenital abnormalities.

The duplication of mutational effects by teratogenic measures is called phenocopy. When similarities in patterns of defects were noted by geneticists and teratologists, the interest in comparative mechanisms of abnormal development ran high. It was hoped that knowledge of the mechanisms by which the teratogen exerted its influence would lead to further information about the genetic basis of comparable abnormalities. The possibility of duplication of genetic effects by teratogens is not impossible, but, because of the extreme complexity of biochemical reactions, growth patterns, and differentiation rates, it is rather unlikely that significant contributions can be made in this direction by teratologists until the approach becomes more biochemical and quantitative rather than descriptive.

Investigations have been made by Zwilling and DeBell (1950) on the patterns involved in gene versus sulfanilimide determined micromelia in chicks and by Landauer (1947) on genetically determined and insulin produced rumplessness in chickens. While the products in both genetically and teratogen-induced abnormalities were rather comparable in both instances, the mechanism of action differed so that there was no indication

of a close relationship of the biochemical pathways involved. Zwilling observed that "it is only through a study of the derangement of premorphological processes that one may actually determine whether different treatments which produce similar conditions do so as the result of non-specific reaction or by specific effects which, at some point, have a common and similar action." Hadorn (1958) stated that "a certain gene-specific sequence of amino acids determined by the template might then be incorporated as building-blocks in different peptides or proteins. From such a mechanism a genuine pleiotropy could be initiated." This observation is of interest here for two reasons; first, we have just recently commented on pleiotropy, and second, because it reminds one of the vast expanse of biochemical reactions which are involved in life at all stages and further indicates that if the one gene -- one enzyme theory is correct, the likelihood of arriving at very basic solutions via teratology is remote. There is some encouragement, however, in Landauer's (1954) observation that teratogens become more specific in their effects as they are applied to the more fully differentiated embryos.

Brief mention will be made of genetic studies in the more widely used and better known experimental animal, the mouse. Gluecksohn-Schoenheimer (1943) presented data on the Sd mutation, a simple dominant, which affects the tail and urogenital system of mice. This particular study is of interest to the current problem because of her analysis of the relationship of ureteral development and metanephric induction. There is a marked similarity in the observations of Gluecksohn-Schoenheimer on urogenital abnormalities in the mouse and the observations of Pohlman (1902)

on similar anomalies in human autopsy material. To these studies could be added a third, the material presented in this paper. Between the three works we find (1) a paper prepared from genetically controlled stock in which renal agenesis is associated with a dominant mutant gene Sd, (2) a paper describing the nature of excretory anomalies of the human which have been called "experiments by nature", and (3) a controlled, but genetically undefined, experiment in teratology which has contributed to the understanding of development and inductive powers resident in the experimental animal the hamster, Mesocricetus auratus.

## SUMMARY

1. The embryo of an albino strain of the golden hamster, Mesocricetus auratus has been shown to be sensitive to hypoxic insult during the 7th through the 10th day of gestation. The array of congenital abnormalities detected is a function of the time and duration of exposure of the maternal organism to the hypoxic insult. The mortality and morbidity rates increased as the duration of hypoxia increased. Within the time period tested, greatest sensitivity of the developing excretory system to the hypoxic stress occurred during the period of most rapid differentiation and morphogenesis which takes place after completion of eight days of gestation. The above results are in agreement with observations of other teratologists on a variety of experimental animals using a variety of teratogens.

2. The nature of the excretory abnormalities ranged from localized deficiencies of the mesonephric duct to bilateral renal agenesis.

3. The mesonephric duct of the hamster appeared to form by elongation of a growing tip rather than by accretion of new cells during its posterior development.

4. The mesonephric duct, accessory ducts from the mesonephric duct, and the ureteric tip were all capable of inducing development of the metanephric blastema.

5. It is proposed that the collecting ducts of the chick mesonephros are homologous with the accessory ducts of the mesonephric duct and the ureteric buds of the mammal.

6. It is suggested that the use of non-pedigreed animals with genetic parameter limited only by the similarity in coat color has possibly afforded a greater variety of effects from exposure to hypoxia than would have been obtained under more closely controlled genetic conditions.

7. It is proposed that the effect of hypoxia may have been related to simple retardation in development by decrease in the amount of oxygen available, accumulation of toxic substances, or interruption of normal placental transfer with possible contamination with maternal substances. Any one of these factors may have interfered with surface-related phenomena involved in cell migration or guidance systems.

8. The abnormalities of development of the mesonephric duct are believed to be due to inhibition of growth and/or differentiation and the incompatibility of localized areas of development with normal mesonephrogenic tissue.

**APPENDIX**

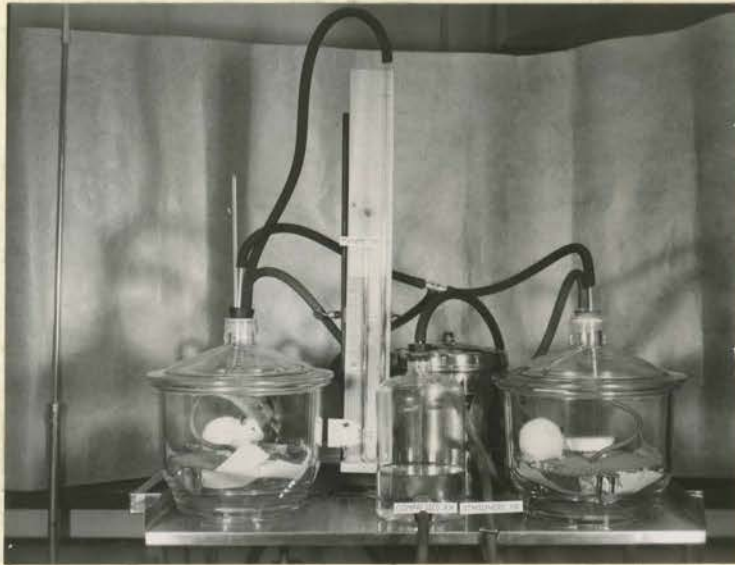


Figure 1

Equipment Used for Production of  
Decreased Atmospheric Pressure



Figure 2

Normal Urogenital Relationships  
of 15 Day Male Embryo (4X)



Figure 3

Normal Urogenital Relationships  
of 15 Day Female Embryo (4X)



Figure 4

Hydronephrotic kidney in Embryo After Ten Hours of Hypoxia on Eighth Day of Gestation (4X)



Figure 5

Unilateral Renal Agenesis (4X)



Figure 6

Bilateral Renal Agenesis (4X)

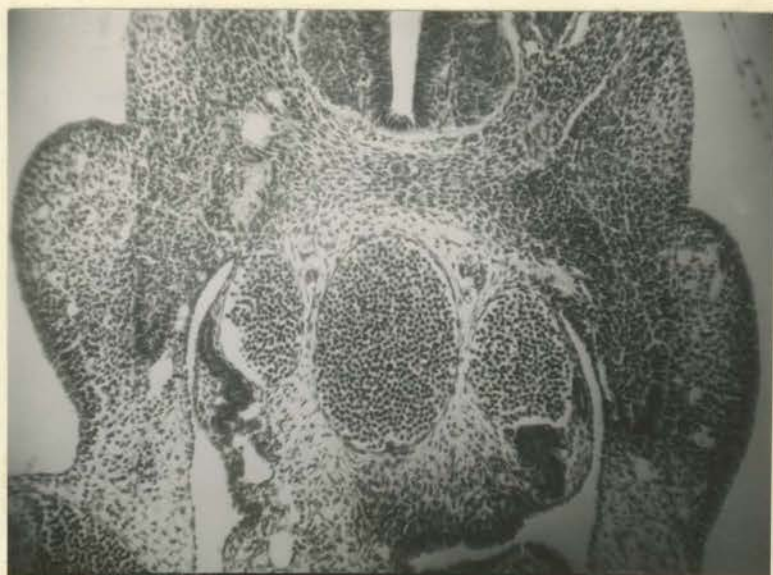


Figure 7

Nine Day Control: Section Through  
Mesonephric Tubules and Ducts (100X)



Figure 8

Nine Day Control: Section Showing Metanephric Condensation  
With Contained Ureteric Bud and Mesonephric Duct  
Relationship with Cloaca (100X)



Figure 9

Nine Day Test: Section Through Embryo 156F Showing  
Cloaca, Metanephric Blastemae, and Abnormally  
Placed Mesonephric Duct (100X)



Figure 10

Nine Day Test: Section Through Embryo 156G  
Showing Continuation of Left Mesonephric  
Duct Beyond Cloaca (100X)



Figure 11

Ten Day Control: Urogenital Ridge Showing  
Mesonephric Tubules and Duct, Mullerian  
Duct, and Gonadal Anlage (100X)

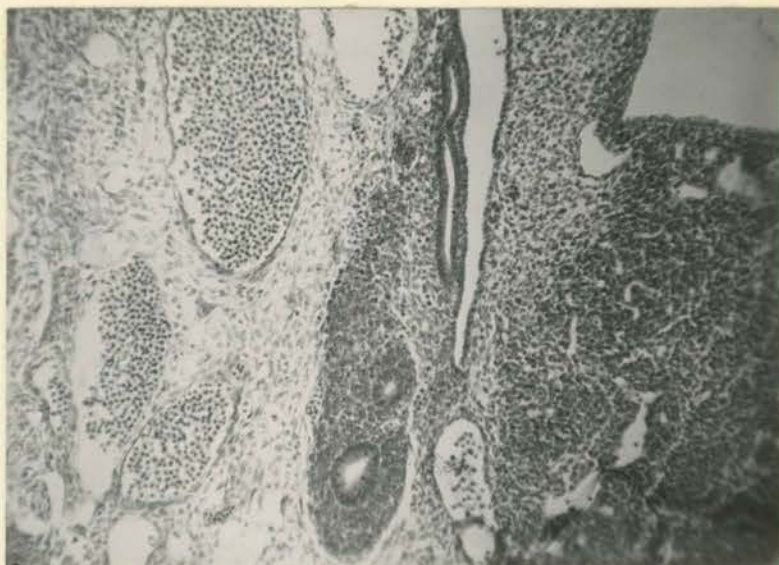


Figure 12

Ten Day Embryo 146G Showing Mesonephric Duct  
and Development of Metanephric Condensation  
about Mesonephric Duct (100X)



Figure 13

Ten Day Embryo 125B Showing Metanephric Centers  
Developing about Accessory Duct (Dorsal)  
and Ureteric Bud (Ventral) (100X)

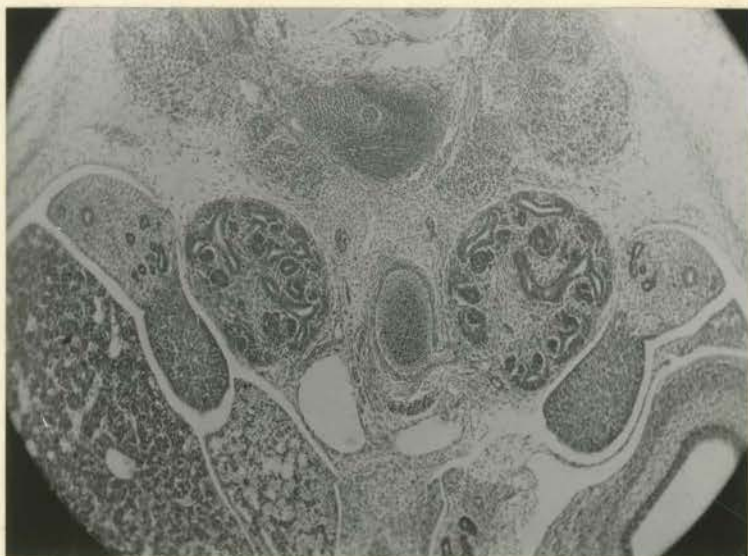


Figure 14

Eleven Day Control: Section Through Urogenital  
Ridges and Metanephroi (50X)



Figure 15

Eleven Day Embryo 172C Showing  
Abnormal Location and Proximity  
of Metanephric Masses (50X)



Figure 16

Eleven Day Embryo 166F Showing Abnormal  
Metanephric Position, Origin of Umbilical  
Arteries, and Major Urogenital Ducts (50X)



Figure 17

Eleven Day Embryo 137A Showing  
Splitting of the Urogenital  
Ridge (50X)



Figure 18

Eleven Day Embryo 137A Showing  
Escape of Mesonephric Duct  
to Body Wall (50X)



Figure 19

Eleven Day Embryo 137G Showing  
Failure of Urogenital Ridges to  
Project into Coelom (50X)



Figure 20

Eleven Day Embryo 137G Showing  
Mesonephric Duct Passing  
to The Dermis (50X)



Figure 21

Eleven Day Embryo 172D  
Showing Mesonephric Duct  
and Development of Meta-  
nephric Condensation about  
an Accessory Duct. (100X)

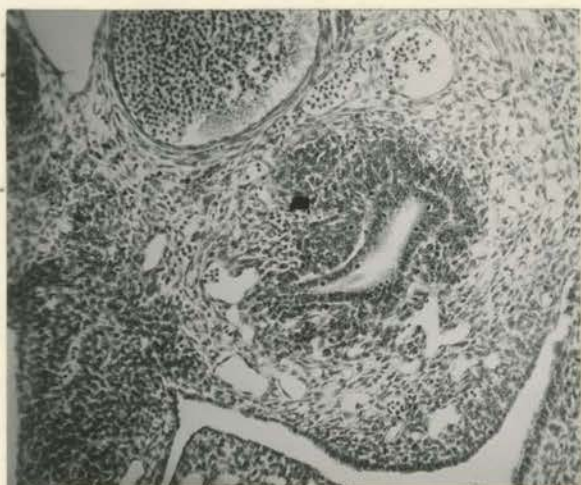


Figure 22

Eleven Day Embryo 172D  
Showing Orientation of  
Metanephric Cells about  
Mesonephric Duct. (100X)



Figure 23

Eleven Day Embryo 172D  
Showing Organization of  
Metanephric Mass about  
Ureteric Expansion.  
(Note mesonephric "ureter"  
ventromedial to true  
ureter. (100X)



Figure 24

Eleven Day Embryo 177C Showing  
Left Metanephros (50X)



Figure 25

Eleven Day Embryo 177C Showing Termination  
of Duct Adjacent to the Cloaca (50X)

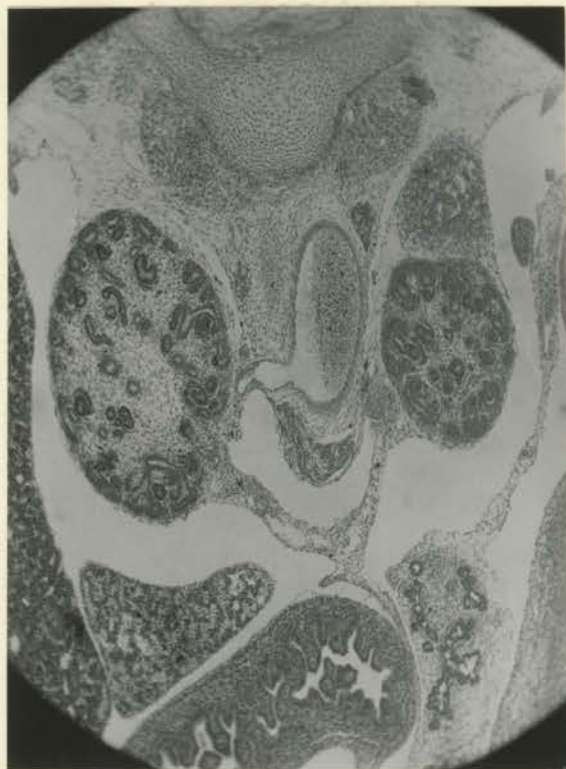


Figure 26

Twelve Day Control: Development  
of Metanephroi (50X)

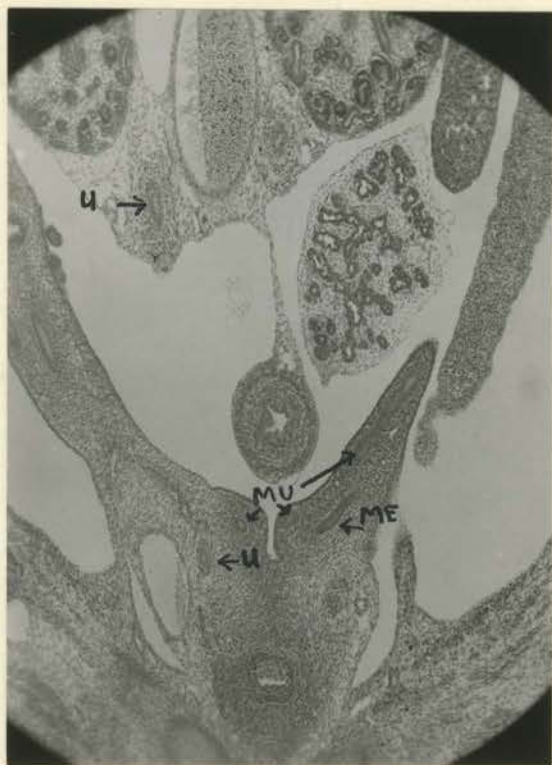


Figure 27

Urogenital Ducts of Twelve Day Control.  
MU., Mullerian Duct, ME., Mesonephric  
Duct, U., Ureter. (50X)



Figure 28

Twelve Day Embryo 131A Showing Left Renal  
Agenesis. (Note Left Metanephric Blastema  
at Arrow.) (50X)



Figure 29

Twelve Day Embryo 131C Showing Origin  
of Accessory Duct from the Mesonephric  
Duct (50X)

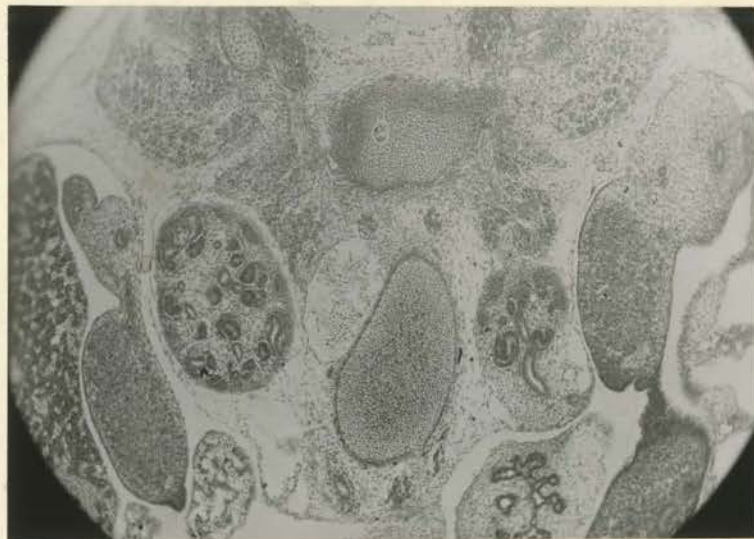


Figure 30

Twelve Day Embryo 131C Showing Entrance of  
Accessory Duct into Metanephric  
Condensation (50X)



Figure 31

Twelve Day Embryo 131C Showing Second Accessory  
Duct (A), Termination of Mesonephric Duct (M),  
and Mesonephric Tubules (T). (50X)



Figure 32

Twelve Day Embryo 142B Showing Two Accessory  
Ducts to Mesonephric Tubules. (50X)



Figure 33

Embryo from Mother Exposed to Five Hours of Hypoxia During 8th Day of Gestation. (2.5X)



Figure 34

Embryo from Mother Exposed to Ten Hours of  
Hypoxia on 8th Day of Gestation Showing  
Herniation of Viscera. (4X)



Figure 35

Eleven Day Embryo 137C to Show Abnormal  
Development of the Spinal Cord. (100X)

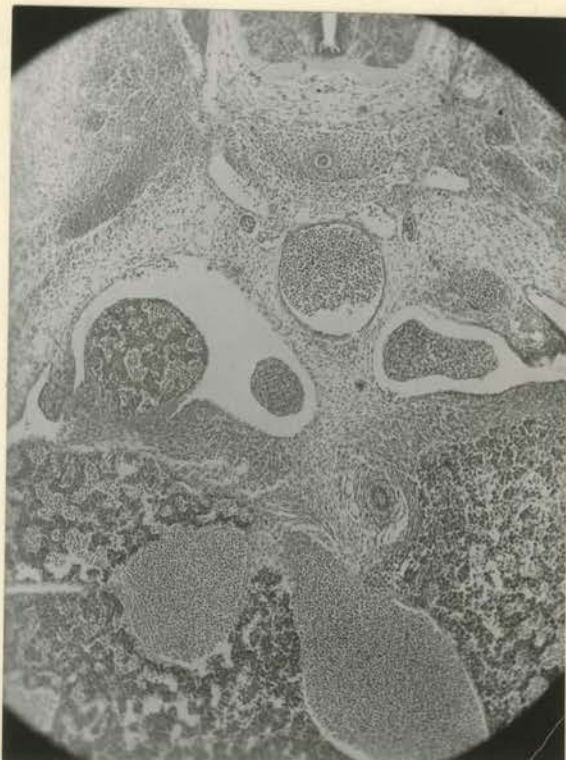


Figure 36

Eleven Day Embryo 172A Showing  
Diaphragmatic Hernia. (50X)



Figure 37

Unilateral Renal Agenesis and  
Two Left Testes. (4X)

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THE EFFECT OF MATERNAL HYPOXIA  
ON METANEPHRIC DEVELOPMENT IN THE HAMSTER

Abstract of a Dissertation

Submitted in partial fulfillment of the requirements  
for the degree of Doctor of Philosophy 1960

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Field of Specialization: Embryology

Major Instructor: Professor Donald I. Patt

Sixty-six non-pedigreed gravid albino hamsters (Mesocricetus auratus) were exposed to a single dose of hypoxia of five to ten hours duration between the seventh and tenth days of gestation in order that the period during which the embryonic excretory system is most sensitive to the action of the teratogen might be determined. Maximum sensitivity occurred during the 10 hour interval between 1:30 - 11:30 a.m. on the 8th day of development (post ovulatory) which is coincident with the period of most rapid differentiation, morphogenesis, and changes in body form (twisting and flexing). The oxygen pressure was equivalent to 40.5 to 48.6 mm. Hg or 25.5 to 30.6 percent of normal atmospheric oxygen. Hypoxia was effected by use of an improvised altitude chamber consisting of two large-sized dessicating jars, a supply of compressed air, a vacuum pump, and a mercury manometer. The reduction of atmospheric pressure was approximately that found at an altitude of 30,500 feet.

Three hundred and five embryos were examined for gross abnormalities after 15-16 days of development. One hundred eighteen embryos were sacrificed between  $9\frac{1}{2}$  -  $12\frac{1}{2}$  days of gestation and examined histologically for abnormalities of the developing excretory system.

The array of congenital abnormalities detected was related to the time and duration of exposure of the maternal organism to the hypoxic insult. Mortality and morbidity rates increased as the duration of hypoxia increased. Abnormalities identified included exencephalocoel, micrognathia, hare-lip, vertebral and rib defects, limb abnormalities, cryptorchidism, renal defects, hydroureter, uterine aplasia, herniated diaphragm, abnormal spinal cord, and one instance of a double testis on one side. The renal

defects included renal agenesis, discontinuity of the mesonephric duct, premature and ectopic termination of the mesonephric duct, development of accessory ducts from the mesonephric duct, multiple metanephric pelves forming about combinations of accessory ducts, mesonephric duct and ureteric buds, and malpositioning of the metanephroi.

Evidence is presented to indicate that the mesonephric duct of the hamster grows by elongation of a growing tip rather than by accretion of new cells from the urogenital ridge during its posterior development. The mesonephric duct, accessory ducts (elongate diverticulae from the mesonephric ducts in areas other than that which normally gives rise to the ureter), and the ureteric tip are all capable of inducing development of the metanephric blastema. It is proposed that the collecting ducts of the chick mesonephros are homologous with the accessory ducts and the ureteric buds of the mammal. The abnormalities of development of the mesonephric duct are believed to be due to inhibition of growth and/or differentiation and the incompatibility of localized areas of development with normal mesonephrogenic tissue.

It is postulated that normal surface related phenomena involved in cell migration or guidance systems have been interrupted. It is proposed that this may be the result of simple retardation of development, inability of the embryo to complete appropriate biochemical syntheses, or accumulation of toxic substances. Alteration in permeability of placental blood vessels might also be a complicating factor.

It is suggested that the use of non-pedigreed animals with genetic parameter limited only by the similarity in coat color has possibly afforded a greater variety of effect than would have been obtained under more closely controlled genetic conditions.

CURRICULUM VITAE



Birth date: February 6, 1928

Parents: Elmer E. Erickson  
Ethel M. (Winch) Erickson

Wife: June Andersen Erickson

Children: Kim Elaine Erickson, 8 January 1953  
John Eric Erickson, 17 August 1954  
Martha Ann Erickson, 28 September 1958

Education:

Elementary School  
Brown School, Watertown, Mass.

**Education (cont.):****Junior High School**

West Junior High School, Watertown, Mass.

**High School**

Watertown High School, Watertown, Mass.  
Graduated, June, 1945

**Higher Education**

Middlebury College 1945-1949  
Bachelor of Arts, cum laude. 1949

Post graduate study.  
Middlebury College 1949-50. No degree.

Boston University Graduate School 1950-51, 1954-55.  
Master of Arts in Biology, 1955

Boston University Graduate School  
Doctor of Philosophy in Biology 1960  
Field of specialization, embryology.

**Military Service****Korean Emergency**

United States Air Force, July 1951-1954  
Served in continental United States  
Highest rank, 1st Lieutenant  
Clinical Laboratory Officer and Medical Supply  
Officer, Tyndall Air Force Base, Florida.

**Reserve Duty**

United States Air Force Reserve 1954-1960  
Highest rank, Captain  
At present, Clinical Laboratory Officer, 619th  
USAF Hospital (Res.)

**Service Schools**

Officer Indoctrination Course, School of Aviation  
Medicine. Gunter Air Force Base, Ala. Nov-Dec. 1951

Hospital Administration for Medical Service Officers,  
School of Aviation Medicine. Gunter Air Force Base,  
Alabama. 1952

Clinical Laboratory Officer School, School of Aviation  
Medicine, Gunter Air Force Base, Alabama. July- Octo-  
ber, 1952.

**Academic Experience.**

Associate Instructor, Boston University College of Liberal  
Arts, 1954-1955.

Instructor, Boston University College of Liberal Arts,  
1955-1960.

**Professional Societies**

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