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Neoplasia and its possible relationship to induction

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NEOPLASIA AND ITS POSSIBLE RELATIONSHIP TO INDUCTION

by

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INTRODUCTION

The proliferation of cells in tissues or organs is a normal occurrence. The cells of the body are always in a state of dynamic equilibrium, for although the cellular constitution of the body may appear anatomically and physiologically constant over a given period of time, the individual cells that compose the body are in a constant state of flux. Cells degenerate; cells die; replacement of somatic cells by mitotic division is constantly taking place. The same cells are not present in animals from their birth to their death (with the possible exception of certain nervous and skeletal muscle cells). A static cellular state does not exist. The units of protoplasm that constitute any given organ are constantly changing; however, the size and functional state of that organ remain the same, within limits.

To maintain constancy within the organism under continued mitosis requires a controlling force. Regular, orderly, and systematic changes could not exist within the organism if a controlling force were not present. The nature of the control is not known at present. However, the control might be effected through the genes within the nucleus or through certain cytoplasmic factors outside the nucleus.

If cells were to divide and proliferate without any control, an orderly state would not exist within the organism. A rapid and chaotic proliferation would produce a cellular growth of unusual proportions. Continued uncontrolled cell division would further enlarge the
mass. Problems of food supply would develop. Food would be obtained at the expense of normal cells. Necrosis of some normal cells would result, and a cancerous or neoplastic mass would be produced.

The controlling force governs cell division and proliferation and also exerts an influence on cellular differentiation, for both proliferation and differentiation must be regulated to conserve order within the organism. A separation of these two fundamental growth phenomena would terminate in a mass of cells that would be undifferentiated. A mass of undifferentiated cells would be a cancerous or neoplastic growth.

Thus, a lack or failure of controlled growth within the organism results in a cellular abnormality manifested by a neoplastic, and sometimes malignant, state.

Within the fertilized egg there exists a controlling force which causes a normal embryo and fetus to be produced. Controlled and regulated growth are demonstrated by the transition from a two-cell to a four-cell stage, and the formation of a blastula, which further proliferates and terminates as a gastrula, which subsequently converges, elongates, and invaginates to produce three germ layers. Invagination produces dorsal mesoderm, which is an induction field, for it establishes axial gradients, areas of organization, and areas that will control the growth of other parts. Subsequent proliferation and differentiation are controlled by inducers or organizers within the embryo.

There are many proofs that a growth-controlling force exists.
within the embryo. Isolation and transplantation experiments have proven that certain areas exhibit growth-inducing, growth-controlling, and growth-promoting factors. Of these areas some exert greater influences on proliferation and differentiation than other areas.

A disruption of the controlling force or organizer or inductor within the developing embryo leads to the production of abnormalities. These malformations may be either tumors, involving one undifferentiated germ layer, or they may be teratomas, involving several undifferentiated or incompletely differentiated germ layers.

Experimentally, abnormalities of a tumorous or teratoid nature can be produced by chemicals (carcinogens, lithium chloride, 2, 4-dinitrophenol, sucrose, and sterols). Experimental abnormalities in embryos can also be produced physically (delayed fertilization, high and low temperature, ultra-violet light, and x-radiation). Such chemical and physical factors disrupt the controlling force within the developing egg or embryo and yield abnormal growths which are in some cases cancerous in nature.

The occasional appearance of tumors or teratomas in feti, without experimental interference, can also be attributed to a disruption of the developmental control or a separation of developmental processes within the embryo. Such abnormalities closely resemble experimentally-produced abnormalities.

The growth-controlling force that exists in the embryo may well exist in the adult. There is no definite proof or evidence to show that this force disappears when the embryo or fetus is completely
formed. Evidence does exist which might lead to the conclusion that growth-controlling and regulating factors are present in the adult. Regeneration, wound healing, tissue repair, and cell replacement are all growth processes that depend on controlling forces for their successful completion.

In adult-tissue regeneration, as exemplified by urodeles, there is a controlled replacement of lost parts. The control is similar to that which exists in the embryo, for it acts on undifferentiated tissue which has formed by a dedifferentiation of adult tissue. The undifferentiated tissue is acted upon by the organizers or controlling factors within the regenerating limb, as the blastomeres are in the embryo. Both regenerating tissue and blastomeres are undifferentiated. They are both acted upon by growth controllers. In the former, they produce a redifferentiation into a new part, while in the latter they form an embryo.

Thus, the cancer problem may be stated as a growth problem. Growth as it occurs in the embryo and in the adult is controlled. Perhaps it is the same growth controller in each case. On the basis of experimental evidence, it can be shown that a disruption of the controlling forces within both the adult and the embryo will lead to a tumor or cancerous growth. Such facts help support the theory that cancer may be produced when residual embryonic control in the adult fails to exert an influence on adult cell proliferation and differentiation. Embryonic inductors, which induce and control proliferation and differentiation in the embryo, may remain in the adult, and, by
their disruption, they may lead to tumors or neoplastic growths in the adult.

A DISCUSSION OF TERMINOLOGY - THE INDUCTOR DEFINED

The terminology used in experimental embryology is vague and in many cases non-descriptive. The main difficulty in choosing terms has been the almost philosophical nature of the field, for at times the uncertainty and intangibility of the subject matter have bordered on the metaphysical.

To speculate or theorize on the nature of a controlling force in cell growth is, indeed, difficult, for the positive isolation of this substance, if it is a chemical and not a physical entity, has not been accomplished beyond all doubt. Therefore, the subject is theoretical and the conclusions reached must be tested experimentally.

The existence of a control has been successfully demonstrated in the amphibian embryo. The action of this controller has been studied and its effects have been observed. Therefore, there can be no doubt that a control exists in the amphibian embryo. However, the substance has eluded isolation. Although numerous and diverse substances with controlling properties have been isolated, these substances may simply activate the growing cells and cause them to release the "natural" inductor. It is also possible that these so called "inductors" may be substances that do not actually exist, as such, within a developing embryo but are merely break-down products produced by experimenters in their attempts to isolate the "actual" inductor. Such substances could
also act by stimulating the release of the "natural" inductor substance.

The terms used to describe the processes of regulation in normal growth, such as differentiation, regeneration, and proliferation, as well as those terms used to explain abnormal growth, are many and, at times, these terms are vague. The ambiguity is due to the elusiveness of the subject matter.

Such terms as controlling force, controlling factor, growth regulator, organizer, organizational field, morphogenetic field, induction field, and inductor have been used in the field of experimental embryology in an attempt to describe the complex cell growth and embryonic growth problems. One factor is inherent in all of these terms, namely, all are attempts to describe the same phenomenon, the ability of some substance to regulate or control the proliferation and differentiation of the blastomeres of the developing egg, so that a well organized and systematically constructed embryo or fetus will result. This substance or factor governs not only cell or blastomere growth; it also governs the type of tissue into which these cells or blastomeres will finally develop. Certain blastomeres eventually form cells that are caused to divide. Simultaneously, these cells are caused to form certain cytological inclusions and characters that distinguish a definite cell type. Certain areas within the embryo, such as the chorda mesoderm, optic cup, lens, or limb bud, play a more active part in the administration of this substance than other more passive locations, such as the ventral ectoderm.
This substance not only controls cell division and cell type, so that they will stay within normal bounds, but it also is the causative agent or inducer in the production of cell division and specific cell types. In addition to acting as a causative and control agent, this substance is influential in performing these functions to a greater extent in some regions than in others. The more active areas can cause or induce the more passive areas to proliferate and differentiate, and then these induced areas can become active and cause other cell growths and differentiations.

It is found that certain terms used in the literature merely indicate a control of proliferation and differentiation in a given cell or large area (controlling force, controlling factor, growth regulator, organizer, organizational field, morphogenetic field). Other terms imply a causation of proliferation and differentiation in a given cell or large area (inductor, induction field).

An induction must be controlled, for merely to cause cell division, without regulating it, would produce abnormalities through wild proliferation. Since it is one substance that apparently causes and controls the formation of an organized entity, a term that implies caused-and-controlled-cell growth and differentiation must be used.

The terms inductor, induction, or induction field are more applicable, for they are more descriptive, and yield a more complete picture of what is actually happening in the embryo. A region is induced to form a differentiated and regulated mass of cells in a controlled manner.
The term inductor will be used to describe a substance that causes a controlled, regulated, and systematic cell growth or proliferation into a definitely differentiated entity. Induction is the process of inducing a cell or area to form a differentiated structure by means of cellular division under the influence of an inductor. An induction field may be defined as an active area in which induction takes place by the action of an inductor.

The other terms, such as controlling force, organizer, and so forth, need not be abandoned. Although they imply control of proliferation and differentiation, and not the starting of the process, it is apparent that the process is set in motion, for, since there is only one substance involved, it must start the action and control subsequent cell division and differentiation regardless of what the term used to describe the process implies. Just as an inductor can induce mitotic division and then control this process, and also regulate differentiation, so can an organizer not only control proliferation and differentiation, but it can also induce or begin the process.

Thus, the various terms possess three important connotations. First, they refer to an ability to start cell growth. Second, they refer to an ability to control mitotic division. And third, they refer to an ability to direct differentiation in a given direction. Since all of the terms describe the same phenomenon, and they all involve these same three points, they may be used synonymously.

THE DISCOVERY OF INDUCTION IN THE EMBRYO

The discovery of an inductor within the developing egg was a
tremendous event in the history of embryology. The demonstration that an inductor existed helped to clarify many developmental mysteries, and it opened the way for further experimental studies.

Two preliminary investigations made this discovery possible. Both involved the introduction of new techniques. The first obstacle that had to be overcome was the lack of a method for determining the fate of the various areas of the normally developing egg. A successful method of transplantation was the second barrier that had to be crossed. After these two obstacles were overcome, the end was accomplished.

Many unsuccessful attempts at determining the prospective fate of egg areas finally led to the successful method of Vogt (1923). At first, attempts were made to remove parts of the developing egg and to note the effects on the resulting embryo. Such experiments proved fruitless, for there were many deaths, and there was a replacement of lost parts. Pricking experiments were likewise resultless, for if an area was damaged mildly it healed and no effects were visible. However, if the pricking was extensive, the results were fatal. Vogt (1923) was the first man to map successfully the normal fate of every part of the developing amphibian egg.

The method used by Vogt was a stain technique. He stained portions of the egg with a vital, non-toxic dye. Thus, he was able to follow development. He applied his dyes (nile blue and neutral red) to pieces of agar and placed the agar in contact with the area of the egg to be observed. He had remarkable success.

Spemann (1920) devised the method of transplantation that was
later used to discover the inductor. His technique permitted the transplanting of any desired part of one developing egg to any part of the same egg or to any other developing egg. He was able to determine by this method the exact fate of a transplanted piece.

Thus, by combining both methods, that is, first knowing what an area will form normally, and then being able to transplant that area into a new location, one was able to determine what effect, if any, the new site would have on the transplanted piece. Thus, Spemann and Mangold (1924) were able to obtain the first induction by an inductor in an amphibian egg, and effect a turning point in the history of embryology.

Dorsal lip of an embryo of Triton cristatus was transplanted into the indifferent ventral ectoderm of an embryo of Triton taeniatus, which was at the same stage of development as T. cristatus. In this new environment it did not act as presumptive medullary plate or epidermis, but it acted on its new location by inducing the formation of a new embryonic axis. Also, neural tube, notochord, auditory vesicles, mesoblastic somites, and Wolffian ducts were induced in the embryo of Triton taeniatus, and therefore, a second embryo was induced on an egg that would have normally formed but one. Spemann (1921) gave the name "organizer" to cells capable of inducing the formation of new anlagen. Spemann (1918) called the region where such cells existed during normal development the "organization-center". Thus, light was cast on the invisible process that occurred during development along with the visible processes of proliferation and growth. The transplanted piece
showed inductoral power, for it induced the cells in its vicinity, which normally do not form neural tube, notochord, and so forth, to develop. Moreover, this transplanted piece also developed into what it would have formed in the old environment if it had not been transplanted. Other induction centers were discovered. Some experimenters investigated the nature of the inductor. Attempts were made to determine its properties, whether it was a chemical, and if so, what type. Further investigations were made into the nature of its liberation, and the location of its storage in the cells of the embryo. From the very first, the nature of the inductor-influence was recognized to be of the utmost importance. Perhaps the inductor would be the key to development.

After Spermann and Mangold had discovered the presence of an inductor within the developing amphibian egg, various theories were proposed to account for this inductoral capacity. The various theories can be grouped into three main categories, as follows:

1. Potential Difference Theory
2. Axial Gradient Theory
3. Chemical Theory

**Potential Difference Theory**

This theory proposes the notion that the process of induction is connected with bio-electrical effects. Hyde (1904) showed that differences of potential existed in different regions of the developing egg, and that these potentials influenced development. He also showed that physiological changes, which depended upon certain definite physical
interactions of electrolytes and colloids, occurred throughout the life-history of the developing egg. He showed that the physiological changes were accompanied by differences of electrical potential, which accompanied progressive changes in ontogeny. Differences of potential were demonstrated by means of a D'Arsonval galvanometer, and by modified capillary electrometers of the Lippmann, Porter, and Lyon types. Such potential differences were demonstrated in the maturing turtle egg and in the fertilized egg of Fundulus.

Hyde (1904) further showed that differences in potential existed between the animal and vegetative poles. He thought that a Helmholtz electric double layer existed between the internal and external surfaces of the egg. These surfaces had opposite electrical charges. The alteration and interaction of the ionic forces within the egg, which produced energy changes, were responsible for the chemical and physical alterations that occurred in the blastomeres, and they also induced the cleavage furrows. Induction was probably due to potential differences.

Spemann's successful induction in Triton taeniatus could be due to potential differences that were established between the transplant and the host. The transplant from the dorsal lip not only has a higher potential (Hyde, 1904), but it also becomes electrically negative in relation to the uncut ventral ectoderm, as is the case in muscle which is cut. Thus, a potential difference could be produced between the dorsal lip and the ventral ectoderm, and the resulting ionic changes would cause induction to occur. Needham, Waddington, and
Needham (1934) expressed some doubt as to the relation of these potentials to induction. They felt that the interpretation of these potentials was an extremely difficult and doubtful task.

**Axial Gradient Theory**

To think of a region as physiologically dominant immediately brings to mind the Axial Gradient Theory. Gradients do exist within eggs. In the frog's egg gradients are established by the unequal distribution of yolk, the unequal distribution of pigment, and the difference in size of the cells in the animal and vegetative poles. The smaller cells are at the animal pole and the larger cells are located at the vegetative pole.

Child (1915) looked upon the physiological axis, in its simplest terms, as a quantitative gradation in physiological action. Gradient is a gradation in the rate of metabolism. The regions of greatest activity or highest metabolic rate are the most susceptible to substances that interfere with metabolism. The areas with the lowest metabolic rate are least upset by metabolic inhibitors. Child (1916) demonstrated that the apical pole is at first most susceptible to potassium cyanide, a metabolic inhibitor, and as development goes on the dorsal lip region becomes most susceptible.

Bellamy (1919) likewise demonstrated gradients of susceptibility to several external agents in the egg and embryo of the frog. The gradients were due to quantitative differences in the protoplasm, and the quantitative differences in the protoplasm were closely associated with differences in the rate of physiological processes. Bellamy
(1919), like Child (1916), found that those parts of the egg in which development first begins, and in which it proceeds most rapidly, die soonest when exposed to external toxic agents. Also, these parts are inhibited most under the influence of various metabolic inhibitors. By such inhibitions, development can be prevented or abnormalities can be produced.

Huxley (1924) noted the similarity between the action in a dominant area and that which is described in the Axial Gradient Theory. He postulated that the dorsal lip, which is a region of most intense metabolic activity, derives its organizing power from this circumstance.

The dorsal lip has a high position on the axial gradient. It has a high metabolic rate. When transplanted, it establishes an area of increased metabolic activity. It is thus a center that could cause a gradient to be established, thus producing changes in its surroundings by alteration of existing gradients and by the establishment of new gradients.

**Chemical Theory**

Induction probably can be explained best on the basis of a chemical substance. This substance would diffuse from cell to cell and exert its influence on cell proliferation and differentiation. The Chemical Theory has had the most support, both from investigators and their experimental findings.

Hangold (1928) studied the quantitative aspect of organizer transplantation, and he made the first relevant contribution to the Chemical
Theory of induction. He found that a large piece of early medullary plate was needed to induce a well-formed secondary embryo. By using a small piece, he found that it had no effect on the formation of a secondary embryo.

Spemann (1931) made the announcement that an organization center retained its ability to induce competent or receptive tissue after the destruction of its cells. Pieces of optic cup, archenteron, and medullary plate, tissues which have the power of induction, were cyto-lyzed by crushing them between two glass plates until only a few complete nuclei remained among the masses of yolk granules. Spemann (1931) found that this disarranged and disrupted material could still call forth the constituent parts of a second embryo.

Marx (1931) showed that the narcotic, trichlorbutylalcohol, had little effect on the organizer. The inductorial power of the dorsal lip was fully retained when its cells were first completely narcotized by trichlorbutylalcohol.

The three experiments noted above are explained best on the basis of the inductor as a chemical substance. However, the Potential Difference Theory can not be eliminated as a possible explanation.

Needham, Waddington, and Needham (1934) were of the opinion that two other experiments (Hamburger and Spemann, 1927, and Spemann and Gegenbauer, 1927) greatly aided the establishment of the Chemical Theory. The explanation of these experiments by either the Potential Difference Theory or the Axial Gradient Theory, in the opinion of these investigators, is extremely vague and doubtful.
Mangold and Spemann (1927) found that a piece of newly-formed medullary plate, if removed and implanted into the blastocoecle of an early gastrula, would induce the formation of a new medullary plate. They called this "homoiogenetic" or "assimilatory" induction. Now this newly formed medullary plate could be implanted into the blastocoecle of another early gastrula, and it would induce the formation of another new medullary plate. Thus, theoretically, the process could be repeated an infinite number of times. This phenomenon of "homoiogenetic" induction pointed strongly toward a Chemical Theory of induction. The Axial Gradient Theory could not account for this result. On the basis of the Axial Gradient Theory, it would be expected that a structure lower down in the axial gradient would be induced and not a structure of equal dominance. On the basis of the Potential Difference Theory, the results of this experiment are still tenable.

Spemann and Geinitz (1927) showed a similar tendency when they "infected" non-inducing cells. A piece of presumptive epidermis, which is normally non-inducing, was implanted into the dorsal lip of the blastopore and was allowed to grow inwards into the roof of the archenteron, and it was then removed. Upon removal, it was implanted into the blastocoecle of a third embryo, and in this location it induced as effectively as normal dorsal lip. Contact with the inductor substance in the induction area of the dorsal lip had caused the non-inducing presumptive epidermis to acquire the power of induction.

These results could be explained on the basis of potential differences or in terms of gradients, but such explanations would be
questionable. The simplest explanation is that of a chemical sub-
stance, which is free in the dorsal lip of the blastopore and in the
medullary plate, and which exerts its influence on tissue with which
it comes in contact.

The cultivation of isolated pieces of embryos produced further
support for the Chemical Theory. Dürenken (1926) and Kusche (1929) im-
planted portions of blastulae and gastrulae into the eye-cup of older
embryos. They observed the production of differentiations, including
notochord and nervous tissue formation. Holtfreter (1929) then de-
veloped a technique for the cultivation of isolated pieces of embryos
in the peritoneal cavity or the lymph-sacs of older larvae of the same
or nearly-related species. A great degree of differentiation was ob-
served under these circumstances. Differentiation occurred even when
the isolates came from undetermined regions of the egg-regions that
were never under the influence of any normal organizer. Presumptive
medullary plate, which is an inductor and which normally would form
neural tissue, and presumptive epidermis, which is not an inductor
and which normally forms epidermis, were both found to form pure
neural tissue.

Holtfreter (1931) obtained different results when he cultivated
embryonic isolates in vitro. Dilute Ringer's solution containing bi-
carbonate exerted no effect on isolates. Ectoderm remained as such
regardless of whether it was presumptive medullary plate or any other
ectodermal structure. It simply formed unorganized epidermal masses.

It was difficult, therefore, to avoid the conclusion that the
in vivo situations were not strictly neutral with respect to inductor power. The implants were reacting to a stimulating substance, perhaps the same substance that normally caused induction. However, this agent was acting by way of the peritoneal fluid and the fluid of the optic cup.

The optic cup has been shown to cause lens formation. The optic cup acts on the ectoderm above it, which is presumptive lens, and induces this area to form a lens. Removal of the optic cup, and the subsequent failure of a lens to form, have helped to establish this notion. Conversely, the removal of presumptive lens results in the formation of a normal eye, for the optic cup induces the remaining ectoderm above it to form a lens after the area has healed.

Wachs (1914) found that isolated pieces of iris formed lens when they were transplanted into the vitreous humor of an eye from which the normal lens had been removed. The agent that has the power to cause lens induction must be able to pass through the medium of the vitreous humor. It must be able to diffuse.

The evidence in support of a Chemical Theory for inductor influence is strong. The presence of an inductor in various areas of the developing embryo can not be doubted. The most logical interpretation of the inductor is that it is a diffusible chemical substance. The exact nature of this chemical substance, that is, whether it be a steroid-like, protein-like, or carbohydrate-like chemical had not been investigated by early workers. Later workers attempted to establish its exact nature.
INDUCTORS IN THE ADULT

The presence of inductors in the adult is a very interesting and valid possibility. It cannot be doubted that some control is necessary in the adult. It is definitely known that control of cell growth exists in the embryo. It has been shown that an interference with, or a disruption of, embryonic control produces abnormal growths, which are in some cases malignant. Neoplastic growths likewise appear in the adult. The characteristics of adult tumors are similar to the features of embryonic neoplasms. Both adult and embryonic tumors exhibit uncontrolled mitotic division and a failure to differentiate. Since the tumors of both the adult and the embryo are similar, and since it is known that a lack of control produces embryonic tumor, then we may assume that a failure of adult control likewise produces a tumor. Perhaps the control is identical in both cases, for the inductor of the embryo may well remain in the adult and there continue to exert its influence on normal as well as abnormal cell division and differentiation.

There is much evidence to support the presence of inductors in the adult. In the adult organism each cell conforms to the character of its surroundings, liver cells continue to form liver cells, heart cells continually differentiate heart cells. Cells cultured in vitro exhibit a tremendous ability to undergo mitotic division. Regenerating tissue also exhibits a subordination of cells within the adult organism to a control by the adult organism.
Witschi (1933) stated that there was a possibility for every cell in the embryo to develop into an inductor within the embryo. He based this statement on the work of Holtfreter (1933), for Holtfreter demonstrated inductorial power in cells of the embryo that were thought not to contain inductor. The factors that suppressed the organizing action were destroyed by heat and dryness, and cells that would not induce normally did so upon exposure to heat and drying. Since every cell of the embryo may possess an inductor, it is possible that this may carry over into the adult. The inductor in the adult may remain in an inhibited state. The inductor may be released by an injury, and it may then function in wound healing or in regeneration to produce a normal replacement of damaged parts. However, this same inductor may be activated in the adult to produce abnormal cell growth.

Weiss (1940) stated that just as there is a constant proliferation of cells from the blastomeres of the egg to the cells of the adult by repeated mitotic division, so there is continuity between the inductorial power of the uncleaved egg and later induction fields. The induction fields produced become more dispersed with continued embryonic cell division, and the inductors are transmitted to all parts of the embryo. The adult receives the inductors as a natural sequence to continued cell division.

Evidence from Regeneration

Regeneration, a process which is found in some adult organisms, is a growth phenomenon which depends upon inductors or organizers. The process of regeneration furnishes, perhaps, the best evidence that
inductors exist in some adult organisms. The ability to replace lost parts is not universally present throughout the animal kingdom. An inverse relationship seems to exist between animal complexity and ability to regenerate lost parts. In general, the more complex an animal, the less is its ability to regenerate. However, there are notable exceptions to this inverse relationship. Ctenophora, which are diploblastic and occupy a low place in evolution, exhibit practically no regeneration. Rotifera, which are pseudocoelus and are not complex phylogenetically, also display practically no replacement of lost parts. On the other hand, such complex invertebrates as the Echinodermata and certain crustacea, which are, indeed, complex anatomically and occupy high places in invertebrate phylogeny, possess remarkable powers of regeneration. In phylum Chordata, regeneration is demonstrated by certain amphibians and even by some mammals. Therefore, regeneration is present in both the invertebrates and the vertebrates. The percentage of regenerators is higher among the lower forms of animals than among the more highly organized forms.

Regeneration is a definite type of development. Weiss (1939) stated that regeneration is possible because developmental factors do not become exhausted when they produce a given structure. The ability of a given structure to form the same part repeatedly is the basis of regeneration. The inexhausted "developmental factors" mentioned by Weiss, may well be inductors, for it was observed that regeneration exhibited controlled cell growth and differentiation, both of these processes being controlled by the inductor of the embryo. Regenera-
tion, which is an adult growth process, may well be governed by the same factor as embryonic growth.

Weiss (1939) stated that regeneration is not a secondarily-acquired process of the organism but, rather, a remainder of the original capacities for cell growth and differentiation through which the individual first came into being. The extent of the ability to regenerate is limited by the extent to which the developmental capacities remain in the animal after ontogeny. The lack of ability on the part of certain more complex forms to regenerate also may be due to their tendency not to lose parts, owing to their protective and defensive capacities.

The difference between wound healing, such as in the epidermis, and regeneration in a newt's tail is quite significant. Regeneration is a complex process involving the complete replacement of lost organs by the action of a growth control on a cell mass (blastema) to induce a growth and differentiation into an organized functioning entity. Wound healing ordinarily involves cellular repair of a damaged area and depends on the capacity of cells to grow and differentiate. However, the cellular proliferation and differentiation in healing usually involves but one cell type and not many cell types as in regeneration. Both processes depend on the adult to exert the inducing influence.

The process of regeneration can be separated into several stages. Butler (1933) described the process in amphibians and summarized the stages as follows:

a) Wound healing - a migration of epithelial cells with little
or no mitosis.

b) Dedifferentiation of cartilage cells.

c) Blastema formation - consisting of altered cartilage cells and mesenchyme cells. Dedifferentiation ceases after the blastema is formed.

d) Histogenesis and morphogenesis by proliferation and redifferentiation of the blastema cells.

Most investigators have found, as did Butler, that the process of regeneration involved several such stages. However, Rose (1944) doubted the blastema formation from cartilage suggested by Butler. Rose maintained that the blastema was formed as a result of epidermal dedifferentiation. He found that young larval skin had a greater potential for regeneration than adult skin. He, therefore, transplanted larval skin in the adult, which does not regenerate, and he observed regeneration. This work of Rose may be questioned, for he obtained the formation of mesodermal structures from the blastema that is formed by the dedifferentiation of epidermal cells that are ectodermal. Thus, ectoderm formed mesoderm, and a new part was produced. This phenomenon needs further testing, for it is unusual to have mesodermal structures (muscles, blood vessels, and connective tissue) formed from ectodermal collagen, even if there is a possibility that the ectoderm is altered.

The question as to which cells actually form the blastema is still unanswered. However, regeneration may be stated as an adult growth process that involves dedifferentiation of certain cells to form a blastema, and to cause reorganization, cell division, and redifferentiation.
regeneration in adult organisms throughout the animal kingdom furnishes evidence that the inductor is present in these animals.

Evidence from Adult Tissue Extracts

Adult tissue extracts have growth-promoting effects on isolated cells in vitro. The ability of such tissue extracts to produce cell growth indicates that these extracts must possess a growth stimulating factor. This stimulator may be a residual embryonic inductor, for the tissue extract exerts the same effect on cells in vitro as embryonic organizer exerts on blastomeres of the partially cleaved egg.

Carrel (1913) found that tissue extracts of the chick embryo, adult chicken, Rous sarcoma, adult dog and adult rabbit caused increased connective tissue growth in vitro. The connective tissue, which was used in these in vitro experiments, was that of embryonic ventricle and skin. Carrel obtained the extracts, which he desired to study, by grinding the necessary tissue together with sand in a mortar. He added Ringer's solution to the grindings and allowed them to stand. Then he centrifuged the solution and used the supernatant fluid.

Carrel (1913) also found that all tissue extracts do not promote growth in vitro to the same degree. Embryonic tissue extract was most active. Extracts of adult rabbit or dog spleen and Rous sarcoma were almost as active as extracts of chick embryo. Heart and kidney extracts were much less active. Extracts of blood corpuscles and of connective tissue brought about only a slight acceleration of growth. Carrel reported that the extract of any adult tissue promoted connective tissue growth. His results indicate that an inductor is present
in adult tissue. It is likewise noteworthy that Rous sarcoma and adult spleen could induce connective tissue growth almost as well as chick embryo extract.

Other investigators confirmed Carrel's results. The somewhat different results reported by a few experimenters may have been due to the difference in their methods of preparing the tissue extracts.

Walton (1914) confirmed the results obtained by Carrel. In addition to confirming Carrel's work, he also demonstrated that liver extract inhibited connective tissue growth in vitro. Liver extract either prevented the proliferation and differentiation of connective tissue cells, or the extract did not possess sufficient inductor to initiate the process.

Trowell and Willmer (1939) found that tissue extracts of the adult chicken had growth promoting properties when tested on chicken fibroblasts cultured in vitro. They confirmed the results of Carrel and Walton.

In the adult mammalian body, as well as in the adult body of other animals, there are certain cells that are not differentiated. However, they possess the ability to become differentiated under proper stimulation. There are "undifferentiated" mesenchymal cells and fibroblasts within the connective tissue of adult mammals. These cells are, to be sure, differentiated into mesenchymal cells and fibroblasts, but these units are arrested developmental structures. They possess the potential to further differentiate under the influence of an inductor. Levander (1945) demonstrated that the mesenchymal cells in adult rab-
bits differentiated when alcoholic extracts of adult rabbit tissue were injected. Alcoholic extracts of bone, muscle, and endometrium induced mesenchymal cells to form bone, muscle, and endometrium, respectively. Levander believed that the same substance was present in the adult tissue as was present in the embryo. He also maintained that embryonic inductor in adult tissue was responsible for post-embryonic growth. Therefore, an extract of a fully developed tissue can cause growth in vivo, for it induces cells to form tissues, and it controls the cellular division of the induced cells.

Although adult tissue extracts can induce cellular proliferation and differentiation both in vitro and in vivo, most experimenters concede that embryonic extracts are more potent in this respect. However, Hoffman, Tenenbaum, and Doljanski (1939a) reported that adult tissue extracts had a greater effect on fibroblasts in Carrel flasks than did embryonic extracts. Equal concentrations of adult tissue extract and embryonic extract had different effects, in that the adult extract induced greater mitotic activity and differentiation than the same concentration of embryonic extract. Hoffman, Tenenbaum, and Doljanski did not give their method of tissue extraction. However, even though their results are not in complete agreement with those of Carrel (1913), Walton (1914), and Trowell and Willmer (1939), the probability of the presence of inductors in the adult is augmented by the results of Hoffman, and others.

Both neoplastic and normal adult tissues have a high growth capacity. Both tissue types contain growth-promoting substances. Hoffman,
Tenenbaum, and Doljanski (1939b) demonstrated that the growth-activating effect of Rous sarcoma extract was not greater than that of extracts of cardiac and smooth muscle of normal adult chickens, when they were applied to fibroblast cultures grown in Carrel flasks (standard technique). Extracts of neoplastic and normal-adult tissues had the same growth-promoting effect. However, neoplastic extract promoted the growth of tumors, while normal tissue extracts promoted normal growth. The sarcoma extract contained inductor, but it was not "normal" inductor. Since the sarcoma extract induced cellular division but failed to produce differentiation, it was unlike the normal inductor contained within adult tissue extract. Thus, the results obtained by Hoffman and others, confirm the theory that adult organisms contain inductors. These results also introduce another concept. The inductor contained within the extract of neoplastic tissue is not functioning "normally", for the result of the induction is an abnormal mass. As noted earlier in this thesis, adult inductors are probably residual embryonic inductors. These adult inductors may produce an abnormal growth, which may be either benign or malignant. Such abnormal growths may well be the result of a disruption of the adult inductor.

**Evidence from Teratoma Testis**

In the testis, the appearance of growths that involve more than one germ layer depends upon two factors: 1) the presence of totipotent cells, and 2) the presence of inductor in the adult. Falin (1940) stated that the inductor was liberated and that it acted on the totipotent cells, and thus produced the teratoma.
Michalowsky (1925) obtained an experimental teratoma in a rooster following the injection of a few cubic centimeters of a five percent solution of zinc chloride into the testis. Falin and Gromzewa (1939) obtained the same result using a ten percent solution of zinc sulphate. Thus, the injection of either zinc chloride or zinc sulphate produced a teratoid growth in the testis of the rooster. Since a growth phenomenon occurred, even though it was abnormal, a growth-prompting agent must have been present.

Falin (1940) proposed an explanation for experimentally-produced teratoma testis. He stated that the zinc reacted with the albumin, which was present in the testis, to form albuminates, and that acids were produced in the reaction. These acids caused tissue injury and necrosis. He observed that there was a close connection between necrosis and the appearance of tumor cells. Therefore, Falin suggested that necrotic cells liberated the inductor and induced the tumor formation. Thus, Falin's theory indicates that the inductor was probably present in the testis of the adult rooster. Since a teratoma was produced, the inductor must have been disrupted.

The appearance of spontaneous teratoma may be explained in a similar manner. In both spontaneous and experimental teratoma an abnormal cell growth is produced. The disrupted or altered inductor, which is liberated, may produce a teratoma by acting on totipotent cells to cause their proliferation and partial differentiation.

Thus, the normal growth processes of wound healing and regeneration depend upon inductors. Also, the evidence from the experiments
with tissue extracts and teratoma of the testes likewise supports the theory that the inductor is present in the adult. Until future experiments definitely establish the presence of the inductor in the adult, caution must be exercised in asserting its existence. At present, the experimental evidence strongly suggests the probability of the existence of inductors in the adult.

**THE NATURE OF THE INDUCTOR**

Although the process of induction may depend upon electrical potentials or metabolic gradients, the majority of the investigators support the theory that the inductor is a chemical substance. There is, however, some disagreement even among the experimenters who assert that the inductor is a chemical substance. Some investigators maintain that the inductor is a steroid-like substance. Others state that it is a substance similar to a carbohydrate. There is also some support for a protein-like inductor. Regardless of whether the inductor is of a chemical, electrical, or gradient nature, its properties are not disputed.

**Properties of the Inductor**

The inductor exhibits many unique properties. These properties are related to its structure. The primary functions of the inductor in the embryo are to establish the body axis, induce neural tube formation, and organize the embryonic germ layers so that an embryo can be formed. The dorsal lip of the blastopore, which gives rise to the chorda mesoderm in amphibians, is thought to be the primary induction
center. Secondary induction centers, such as the optic cup and the limb bud primordia, emanate from the primary induction center. The main attribute of the inductor within the embryo is its organizing ability.

Child (1946) stated that the inductor substance was non-specific, and that it was widely distributed. He based this statement on the observation that chick, fish, and lamprey inductor caused neural differentiation in the ventral ectoderm of the amphibian. Thus, the ability to induce neural tube formation is not restricted to the inductor of any one animal type. The inductor can exert its effect on many animals. The inductor is non-specific in both distribution and action.

Some investigators have shown that the inductor was extremely stable in a wide temperature range. Bautzmann, Holtfreter, Spemann, and Mangold (1932) found that by removing dorsal lip from an amphibian egg and heating the lip to 60°C., it did not lose its inductive power when it was transplanted into the ventral ectoderm of a second embryo. Holtfreter (1933) stated that heating amphibian dorsal lip at 60°C., or boiling it for five minutes, failed to reduce its inductive power. It was also reported by both Holtfreter (1933) and Bautzmann and others (1932) that freezing temperatures, likewise, failed to destroy the inductive potential of chorda mesoderm. Waddington (1933) showed that the anterior portion of the chick's primitive streak, when coagulated by heat, and when implanted within the blastoderm of a host, induced neural plate and notochord formation. From the experiments noted above, it would appear that the inductor is stable to heat. However, other investigators do not support this view, as will
be shown under "The Liberation of the Inductor".

Some experimenters have asserted that the inductor was stable when exposed to certain chemicals. Tissue that was known to possess the inductor did not lose its ability to induce when exposed to ethyl alcohol or trichlorbutylalcohol. Bautzmann and others (1932) demonstrated that inductoral power was not lost when dorsal lip of an amphibian egg was placed in ninety-six percent ethyl alcohol for several minutes. Marx (1931) demonstrated that the narcotic, trichlorbutylalcohol, did not prevent amphibian dorsal lip from inducing neural tube formation after the dorsal lip had been completely narcotized.

The inductor can be liberated in many ways. The boiling, crushing, or drying of ventral ectoderm of the amphibian egg apparently stimulates this region to release the inductor. In the development of the amphibian embryo, ventral ectoderm does not act as an inductor. Thus, Child (1946) stated that some tissue liberated the inductor when living, while other tissue yielded the inductor only when dead. Such violent treatments as boiling, crushing, and drying kill the ventral ectoderm tissue, but they release the inductor. Likewise, Holtfreter (1933) found that parts of the egg that had no inductoral power when living, could acquire it after being boiled. He found that presumptive epidermis of the gastrula, or ventral epidermis of the neurula, when dead, called forth inductions as strong as those induced by living medullary plate. Fertilized uncleaved amphibian egg also induced when it was first boiled. Therefore, another property of the inductor is its ability to induce when "dead".
With the reservations noted in the above discussion, the properties of the inductor may be summarized as follows:

1. The inductor possesses the ability to organize an embryo.
2. The inductor is widely distributed throughout the animal kingdom.
3. The inductor is non-specific in its action.
4. The inductor is stable to both boiling and freezing temperatures.
5. The inductor is stable to certain chemicals.
6. The inductor can induce when "dead".

**A Steroid-like Inductor**

Sterols, or lipids, are soluble in certain fat solvents, such as chloroform, ether, and petroleum ether. Needham, Waddington, and Needham (1934) demonstrated that induction of a secondary embryonic axis in amphibian gastrulae was accomplished by the implantation of ether extracts and "petrol-ether" extracts of the neurula and ether extracts of adult amphibian viscera. Therefore, they concluded that the inductor was probably a definite chemical substance soluble in ether or "petrol-ether". Since ether and petroleum ether are fat solvents, it seems, from the above experiments, that the inductor may be a sterol substance.

Waddington, Needham, Nowinski, and Lemberg (1935) obtained an inductorial substance, which was capable of causing the ectoderm of the Triton gastrula to differentiate into neural tissue, by making ether extracts of whole newt bodies and mammalian liver. The active sub-
stance was present in the unsaponifiable fraction and in that part of
the fraction precipitable with digitonin. An inductor and cholesterol,
which is a complex, colorless, crystalline solid alicyclic alcohol,
were precipitated when the unsaponifiable fraction was allowed to
crystallize from alcohol in the cold. Waddington and others suggested
that the inductor substance was of a sterol-like nature.

Barth (1934) reported that the cephalin, which is phosphorized
fat obtained from mammalian brain, induced a neural plate formation in
axolotl. He also reported that the extract of mammalian brain, used
to obtain neural plate, contained impurities which were probably
lecithin or cholesterol. It was possible that the impurities produced
the induction. However, he asserted that cephalin had many properties
of the amphibian organizer. Thus, Barth's results lend support to a
chemical theory of induction, wherein the inductor is probably a
sterol-like substance.

Waddington and Needham (1935) stated that the inductor was
probably an unsaponifiable ether-soluble substance. They based their
statement on experimental results, for they tested the effects of
sterol substances on amphibian gastrulae and found that induction took
place. Into the cavities of young amphibian gastrulae, they implanted
small pieces of coagulated egg-albumin which contained, in an emulsified
form, the substance to be tested. They experimented with hydrocarbons of the phenanthrene group that are ether-soluble and unsaponifiable. Since compounds which belonged to the phenanthrene group induced neural tube formation, there is a strong possibility that the
naturally-occurring inductor is a sterol-like substance.

Woerdeman (1933) concluded that the inductor was associated with glycogen and was probably a carbohydrate-like substance, for inductions were obtained with glycogen extracts. Waddington, Needham, Nowinski, and Lemberg (1935), however, isolated from crude preparations of glycogen an active ether-soluble substance which was also precipitated with digitonin. They suggested that the inductor-power of glycogen was due to the presence of sterol impurities. Waddington, Needham, Nowinski, Lemberg, and Cohen (1936) confirmed the presence of an inductor substance in the digitonin precipitate of the unsaponifiable material of the ether extracts of crude glycogen. They, therefore, stated that glycogen was not capable of inducing neural tube formation in amphibian gastrulae. They also stated that the induction, apparently caused by glycogen, was actually due to sterol contaminants.

The experimenters mentioned above presented evidence that the inductor was probably a sterol-like substance. However, as discussed below, other investigators suggested that the inductor was carbohydrate-like or protein-like, and they supported their theories by evidence equally as strong as that cited above.

A Carbohydrate-like Inductor

The theory that the inductor is carbohydrate-like is based on two observations. First, glycogen, which is a type of carbohydrate, is capable of inducing neural tube formation in amphibian gastrulae. Second, the metabolism of glycogen is associated with induction areas.
A carbohydrate product released in the metabolism of glycogen, or some other substance related to glycogen metabolism, may be the agent responsible for induction.

Woerdeman (1933) found that the glycogen content was high during the amphibian blastula stage but, as gastrulation occurred and invagination took place, there was a decreased glycogen content within the developing egg. He contended that the loss of glycogen was associated with a decreased inducing capacity. The inductor was a carbohydrate-like substance, and glycolysis was the means by which the inductor performed its functions. Woerdeman further substantiated this theory with evidence from tumor inductions. He used Walker rat carcinoma 256, a malignant tumor, which has a high glycolytic activity and obtained a positive induction. However, inductive capacity does not always depend upon a high glycolytic activity. Wehmeier (1934) reported that the retina of the frog, a tissue of high glycolytic activity, was an extremely weak inductor. Since there was some induction produced with the retina, the carbohydrate-like theory of the inductor was not disproved. However, the view needed further confirmation.

Fischer and Wehmeier (1933) stated that induction was caused by increased glycolysis and that the content of glycogen present within the developing egg influenced the inductorial capacity. They helped confirm the findings of Woerdeman (1933), for they obtained inductions with glycogen.

Boell (1942) stated that induction in the amphibian occurred in that region of the gastrula characterized by a prevailing metabolism
of carbohydrates. He, likewise, maintained that the inductor was either a carbohydrate-like substance, or that it was intimately associated with carbohydrate metabolism.

Holtfreter (1933) was unable to confirm the results that glycogen could induce neural tube formation in the amphibian. Jaegar (1945) stated that neither glycogen nor any of its metabolic products was essentially concerned with induction.

Thus, the ability of glycogen to induce is questionable. However, the carbohydrate theory is strengthened by an investigation of the metabolism of glycogen. The rate of metabolism, as postulated by Child (1915), is higher in the dorsal lip than in the ventral ectoderm of the amphibian egg. In order to maintain a high metabolic rate, a tissue must consume much oxygen. Woerdeman (1933) demonstrated that the glycogen content was higher in the dorsal lip than in the ventral ectoderm of the amphibian egg. Thus, the metabolism of glycogen might be related to induction, for a high metabolic rate and a large glycogen content exist in an induction field.

Brachet and Shapiro (1937) studied the oxygen uptake of Rana sylvatica embryos by means of the Gerard-Harttine microrespirometer. They showed that the dorsal lip area respired at a consistently higher rate than the ventral region. The average difference was forty-seven percent. Similar experiments performed on the unfertilized eggs of Rana pipiens showed no significant difference between the animal and vegetative poles. Increased oxygen uptake by the dorsal lip of the fertilized R. sylvatica egg indicated increased metabolic activity.
The probability that the increased oxygen uptake favored the metabolism of glycogen was great. The further possibility that glycogen is concerned with induction is somewhat increased.

Boell, Needham, and Rogers (1939) utilized the Cartesian diver ultramicromanometer to study the metabolism of amphibian gastrulae. They found that the dorsal blastopore lip, which is an induction center, showed anaerobic glycolysis and anaerobic ammonia production three times as high as those of the ventral ectoderm. These results confirmed the findings of Brachet and Shapiro (1937). The inductor was related chemically to glycogen or was carbohydrate-like, and it was present in a region of high metabolic activity.

The respiratory quotient is an index of the type of food that an animal or a tissue is utilizing. Boell, Kock, and Needham (1939) demonstrated that during the gastrulation of the amphibian, Amblystoma, the dorsal lip region showed greater trend toward a respiratory quotient of unity than did the ventral ectoderm. The respiratory quotient of the ventral ectoderm rose as development progressed, but Boell and others doubted whether the quotient ever reached unity before the ventral ectoderm was completely underlain with mesoderm. They obtained these results by using the Cartesian diver ultramicromanometer. A respiratory quotient of unity indicated that carbohydrate was metabolized. Thus, the theory that the inductor was carbohydrate-like was supported. The metabolism of carbohydrates took place in the induction field of the dorsal lip. The inductor, which was present in this area, may well have been a carbohydrate.
Barth and Graff (1938) stated that since the organization center of the amphibian egg had a higher rate of oxygen uptake and anaerobic glycolysis than the remainder of the egg, the inductor was liberated in the induction field during the metabolism of glycogen. Therefore, the inductor was probably of a carbohydrate-like nature.

It should be noted that all investigators did not substantiate the glycogen-metabolism, carbohydrate-like inductor theory. Boell and Needham (1939) reported that the respiratory rates of the dorsal lip and ventral ectoderm regions of the gastrulae of Discoglossus and Amblystoma appeared to be identical. Waddington, Needham, and Brachet (1936) also stated that the measurements of the oxygen-uptake of the dorsal lip of the blastopore and of the ventral ectoderm by a microrespirometer indicated that only a very small difference, if any, existed between the respiratory rates of these two regions in the developing amphibian egg. These results agreed very well with those of Boell and Needham (1939), who used a Cartesian diver ultramicromanometer to determine the respiratory rates of the dorsal and the ventral areas of the amphibian egg. The findings of Boell and Needham (1939), as well as those of Waddington and others (1936), indicated that the oxygen uptake of the induction-field area of a developing amphibian egg and that of a non-inducing area of the same egg was similar. Also, these findings indicated that the release of a carbohydrate-like inductor in the induction area by metabolism was extremely doubtful.

Barth (1942) stated that the discrepancies in the literature pertaining to oxygen consumption of amphibian gastrulae are due to two
factors. First, the investigators did not always state specifically which portion of the ventral area of the gastrula was used. Second, most investigators did not correct for the oxygen consumption of the yolk. Barth found that the dorsal lip consumed oxygen at a higher rate than the opposite side of the amphibian gastrula, since he corrected for the yolk's consumption of oxygen. His results agreed quite well with those of Brachet and Shapiro (1937). He found that his results could not be compared with those of Waddington, Needham, and Brachet (1936) and Boell and Needham (1939), since these experimenters did not use that region of the gastrula directly opposite the dorsal lip but used some other portion of the ventral ectoderm. Furthermore, Barth found that when he did not correct for the oxygen consumed by the yolk, he, too, obtained the same rate of oxygen consumption for the ventral ectoderm and the dorsal lip. Thus, Barth concluded that the inductor was carbohydrate-like and that it was associated with metabolism.

Barth (1938) demonstrated that oxygen was also important in regeneration. He stated that oxygen was essential to the regenerative process, since it functioned to liberate the inductor. He found that a decrease in the available oxygen supply caused a decrease in the rate of regeneration in Tubularia. Increased oxygen tension above four and one-half cubic centimeters per liter increased the rate of regeneration. Oxygen tension lower than this level decreased the rate of regeneration. A direct relationship existed between oxygen tension and regenerative rate.
Metabolism may be important in regeneration in the same manner as it is in ontogeny. The liberation of the inductor in both regeneration and in development may depend upon metabolism. Since the oxygen tension affects the rate of metabolism, the release of the inductor may also be affected. It is probable that in both regenerative and developmental phenomena the oxygen aids the process of glycolysis that is taking place. Therefore, an inductor that is probably carbohydrate-like may be present in the adult and it may be liberated through metabolic processes during regeneration. It may be maintained by some that there is evidence sufficiently strong to support the theory that the inductor is carbohydrate-like. However, this theory has not had the complete support of all investigators.

A Protein-like Inductor

The theory that the inductor is a protein-like substance is supported by some investigators. However, it is difficult to explain certain properties of the inductor if it is assumed that the inductor is a protein. As noted earlier in this thesis, the inductor is presumably stable to high and low temperatures. Proteins are not stable to high and low temperatures. Also, the inductor can supposedly induce after it has been crushed or dried. Proteins cannot maintain their molecular structure under these conditions. On the other hand, proteins are specific in their action. The inductor induces a specific structure to form, for example, a lens, a nerve tube, or a notochord. Inductors transplanted into a definite area exert a specific influence. Optic vesicle, transplanted into the ventral ectoderm of the same or a dif-
ferent egg, causes lens formation in the new location. Dorsal lip is also specific when transplanted, for it induces the same structures in the transplanted area as it would have produced if it were not transplanted.

Barth and Graff (1938) stated that the sterol theory of the organizer was not correct. They concluded that a sterol-like inductor lacked the specificity required of an inductor. Fat and fat-like substances were not specific in action. However, proteins were specific. Therefore, they concluded that the inductor was a protein-like substance.

The specificity of the inductor is reflected in tumor transplants. Berrill (1943) stated that the fact that tumor transplants from one species were incompatible with the tissues of other species, indicated that the inductor was a protein-like substance. A host that received a foreign tumor transplant lacked the inductor to control the transplant. Such transplanted tumors were either resorbed, or they took and proliferated wildly. Both a resorption and a successful transplant that grows chaotically exhibit a lack of control by an inductor. The prevailing lack of control is due to tissue specificity. Only a protein-like inductor could exhibit such specificity.

Briggs and Grant (1943) demonstrated that carcinoma of adult *Rana pipiens* kidney transplanted into various sites of young larvae grew well. However, when these host larvae approached metamorphosis, the tumors regressed. The tumor regression was not due to metamorphosis, for permanent tadpoles, and tadpoles which had been hypophysecto-
mized, also underwent tumor atrophy. Briggs and Grant (1943) stated that the atrophy was an expression of development. The younger larvae lacked the protein-like inductor, for their tissues were not differentiated. However, as the larvae grew older, their tissues became specific because of the presence of the protein-like inductor -- regression occurred.

Dürken (1926), Kusche (1929), and Bautzmann (1929) indicated that fragments of blastulae and gastrulae developed without disturbance in the coelom, optic cavity, and dorsal lymph spaces of older amphibian larvae. They reported that the transplant did not regress. However, they failed to report the age of the host.

Harris (1941) demonstrated that presumptive ectoderm, notochord, and entoderm from gastrulae of *Hyla regilla*, when transplanted into the optic cavity, dorsal lymph spaces, brain cavity, and muscles of the tail of *H. regilla*, underwent regression. This regression occurred in older tadpoles with well defined limbs. Harris stated that there was a difference between the cells of the host and those of the graft, and consequently regression occurred. At first, the host was not sufficiently unlike the graft, so that the grafts were able to grow. As the host grew older, it differentiated further. The protein-like inductor present became more estranged from the graft. The inductor of the host failed to exert its influence on the transplant, and the transplant regressed. Harris (1941) stated that there was no evidence of a sarcoma-like growth resulting from the lack of control; there was a simple regression. However, Dürken (1926) obtained re-
sults which were not in agreement with those of Harris (1941). Dürken reported an atypical mesenchyme or sarcoma-like growth as a result of the failure of the host to exert a control on the graft.

Thus, there is strong evidence from the experiments with adult tumor and embryonic transplants that the inductor is probably protein-like.

Barth (1939) stated that the inductor was protein-like. He asserted that the subjection of the dorsal lip to heat, drying, and alcohol treatment by Holtfreter (1933) denatured the protein-like inducers. The specificity of induction was lost. Barth (1939) asserted that the induction by denatured dorsal lip, reported by Holtfreter (1933), was due to the release of the natural protein-like inductor that was stimulated to action by the denatured protein. Barth (1939) also stated that in Amblystoma, proteins had a greater capacity to induce neural tube formation than did lipoid material.

Brachet (1939) suggested that natural inductors are proteins. He also suggested that the natural inductors were present in the cell nuclei. He stated that the inductor might be either a nucleoprotein or a nucleic acid. Sinnott (1939) stated that the genes were important in induction. Thus, he, too, confirmed the theory of Brachet (1939) that the inductor was probably located in the nucleus and was of a protein nature. Brachet (1947) concluded that the inductor was a protein-like substance and that it was a nucleoprotein complex that contained respiratory and hydrolytic enzymes.

There is a definite possibility that the inductor is protein-
like. However, there are certain facts that tend to cast some doubt on the protein-like nature of the inductor. High and low temperatures cause proteins to become denatured. These proteins are altered in molecular structure and lack specificity. Chemicals, likewise, cause the denaturing of proteins. Furthermore, Child (1946) stated that the nature of proteins was such that they would have little chance of surviving the extreme conditions of temperature and chemical treatment that chorda mesoderm had been exposed to and still survived. Child concluded that it seemed unlikely that the natural inductor was a protein type.

However, the protein-like theory of the inductor should not be discarded. To be sure, heat, cold and chemicals may kill the dorsal lip tissue of the amphibian gastrula. The subsequent induction may not be due to "living" inductor but to the ability of the "dead" inductor to activate the natural inductor in the region where the denatured dorsal lip is applied. Therefore, the natural inductor may well be of a protein nature.

Thus, the cases for all three possible chemical types of inducers have been elucidated. Harrison (1933) warned against singling out any one chemical substance as the active agent in the production of proliferation and differentiation. He may well be correct, since the evidence for any one chemical substance, as reported in the literature, is not substantiated by all experimenters, but it is invariably contradicted by some. Hence, the exact nature of the inductor is still in doubt.
THE LIBERATION OF THE INDUCTOR

The inductor must be liberated from such areas as the dorsal lip, optic cup, or limb bud in order to exert its influence. An inductor that is inhibited physically or bound chemically can not function to produce cell division and differentiation. The inductor can be liberated in many ways. It has been asserted that cell injury, metabolism of cells, action of carcinogens on cells, and cellular cytolysis are all mechanisms in causing the liberation of the inductor from an area in which it is contained.

As noted earlier in this thesis, there is a supposition by some investigators that the inductor is stable to high and low temperatures, drying, and chemicals. The inductor is assumed to be capable of inducing even after receiving injurious treatments that should destroy its inductorial power. Other investigators believe that the property of the inductor to induce when "dead" is questionable. There are two possible explanations for the ability of dead tissue to induce. First, the killing of non-inducing tissue by boiling, or by any other means, causes the liberation of inhibited inductor within the tissue, and this released inductor promotes mitotic division and differentiation. Second, the destruction of either non-inducing or inducing tissue by heat, or by any other means, causes the liberation of a substance which, in turn, stimulates the release of the "natural" inductor.

Needham (1942) stated that boiling, or other treatments that killed tissue, served to liberate the inductor from all regions of the embryo, even from those areas which did not induce before the treat-
ments. He also stated that such treatment of ventral ectoderm caused this area to acquire inductive power, because a natural inductor was liberated. The inhibition was removed by the treatment. Holtfreter (1933) asserted that non-inducing ventral ectoderm of the amphibian gastrula did induce after being treated by physical means or with chemicals. Tissue treated in such a manner and implanted in a second amphibian gastrula evoked neural tube formation in the host gastrula. Needham (1942) explained these results on the basis that the natural inductor was liberated from the ventral ectoderm by the heat treatment and that the liberated inductor produced the neural tube formation.

Child (1946) stated that methylene blue, when applied to ventral ectoderm of an amphibian gastrula, caused this tissue to induce neural tube formation in the ventral ectoderm of another amphibian gastrula onto which it was implanted. Needham (1936) maintained that although the dye produced a metabolic acceleration, the acceleration was low. Therefore, he concluded that the effects produced by the substance were due to the liberation of restrained inductor from the ventral ectoderm. The induction produced in the second amphibian embryo was due to the release of the natural inductor. The methylene blue was not the actual inductor, but it served to liberate the inductor. Weiss (1935) likewise stated that the various chemical and physical treatments merely activated the inductor. The activated inductor produced the subsequent inductions directly.

Inductions have been produced in ventral ectoderm by treatment with other chemical substances. Beatty, de Jong, and Zielinski (1939)
found that the dyes Janus green and neutral red caused pieces of presumptive epidermis, ventral ectoderm of the amphibian gastrula, to undergo neural differentiation. Okada (1938) stated that, in the embryos of *Triturus pyrrhogaster*, introduction of the mineral materials Fuller's earth, silica, and calcium carbonate led to inductions of neural tissue in the ventral ectoderm. Since the ventral ectoderm was treated with many substances, and since, when it was so treated with these irritants, the non-inducing area was induced, the inductor probably was liberated in its natural form. Child (1946) concluded that the apparent inductorial power of many substances was due to their effectiveness in liberating the natural inductor.

A sterol-like or a glycogen-like inductor may be able to withstand such treatments as discussed above. However, a protein-like substance certainly cannot withstand such violent treatment. The direct liberation of a sterol-like or glycogen-like inductor by chemical or physical means is probable, while the direct liberation of a protein-like inductor by such means is impossible.

It is possible, however, that a sterol-like, a glycogen-like, or a protein-like substance could be liberated indirectly from ventral ectoderm tissue in which it was located. Use of heat, drying, ethyl alcohol, silica, and so forth, seems to kill the tissue and destroy the natural inductor. The destroyed inductor subsequently may act on intact cells that are stimulated to release the natural or intact inductor.

Barth and Graff (1938) stated that neural induction was a response
to a stimulus. They also believed that the stimulus could be supplied by the action of physical or chemical means on ventral ectoderm to produce dead cells. The dead cells produced toxic substances that stimulated living cells. The living cells then liberated the inductor substance. They further stated that there was no absolute proof that the dead cells of the ventral ectoderm, produced by heat, for example, contained the natural inductor.

Cohen (1938) substantiated the conclusions of Barth and Graff (1938) by experimental evidence. He injured cells of the developing amphibian egg and observed neural tube formation. He inserted a microcautery needle into the blastocoel of young gastrulae of Rana pipiens. However, he did not inject any chemicals. As a result of the cauterization, protuberances appeared over the injured area. The rounded projections had folds and lumen and gave the appearance of medullary plate and neural tube. Injury resulted in dead cells that stimulated living cells to release the inductor.

Holtfreter (1945) stated that in most cases reported in the literature, in which dead cells or foreign substances "induced" development, the effects produced by these cells may be regarded as indirect. The action was effected by the cytolyzed cells of the host. The destroyed cells liberated substances that acted on intact cells and caused the normal cells to liberate the inductor. Thus, the inductor may be liberated indirectly by the products of cellular destruction.

In the above discussion it was shown that the inductor may be liberated as the result of an injury. The injury was produced ex-
experimentally. However, the inductor is also liberated naturally, without injury. The inductor may be liberated as a metabolite or by the process of metabolism. Induction areas, or areas that are known to possess the inductor, show a higher oxygen uptake than non-inducing areas, when the oxygen consumed by the yolk present in the ventral region of the egg is taken into consideration. The process of metabolism has usually been associated with carbohydrate-like inductor. Regardless of the chemical nature of the inductor, that is whether it is a sterol, protein, or a carbohydrate, it may well be associated with metabolism and enzyme systems, since all three types of chemical substance are liberated by metabolic processes.

Beatty, de Jong, and Zielinski (1939) found that Janus green and neutral red accelerated cellular respiration and caused ventral ectoderm of the amphibian gastrula to form neural tube. The dyes increased the metabolic rate. Thus, they may have been able to liberate the inductor as a metabolite, or they may have increased the functional capacity of the inductor by increasing the oxygen uptake.

Brachet and Shapiro (1937) stated that the rate of oxygen consumption of the dorsal region of the gastrula of Rana sylvatica was forty-seven percent higher than that of the ventral region. Boell, Needham, and Rogers (1939) also stated that the induction field of the amphibian egg exhibited an oxygen uptake that was three times as high as that of the non-induction area of the ventral ectoderm. Barth (1942) gave further support to the theory that the inductor was essentially concerned with metabolism. He, too, reported that the dorsal
lip of the amphibian gastrula consumed oxygen at a higher rate than did the ventral ectoderm. Brachet (1947) likewise stated that the inductor was present in certain areas of the developing embryo as a consequence of metabolic processes. He also stated that the inductor was liberated by enzyme action during metabolic processes. Thus, the evidence in support of the theory that the inductor is liberated by a metabolic process is strong.

The inductor can also be liberated from the developing amphibian egg by certain carcinogens. Shen (1939) reported that the water-soluble carcinogenic hydrocarbon, 1:2:5:6-dibenz-anthracene-\(\Phi\)-indosuccinate, produced neural tube formation in the gastrulae of Triton alpestris. The hydrocarbon was implanted in the ventral ectoderm. Shen found that the optimum number of neural tubes was induced by a dose of 0.0125 gamma of hydrocarbon per embryo. Concentrations of this hydrocarbon, higher or lower than this value, resulted in a decrease in the percentage of successful inductions. He asserted that if the successful inductions were due to injury, as other investigators maintained, the higher the dosage of hydrocarbon, the greater would be the injury and the higher would be the percentage of successful inductions. However, he maintained that, since there was an optimum dosage of hydrocarbon for optimum induction, it seemed unlikely that an injury resulted from the administration of the hydrocarbon. Consequently, according to Shen, an injury did not cause the release of the inductor when this hydrocarbon was used.

Shen also stated that the dose of water-soluble hydrocarbon that
produced the maximum effect on the ectoderm of the gastrula was in the same range as many other biological stimulating substances. On this premise, Shen concluded that this hydrocarbon, and other hydrocarbons that are active in neural induction, exhibited a direct action on the ventral ectoderm. The water-soluble hydrocarbon, like the normal inductor, acted directly to evoke neural tube formation and did not act by liberating the masked inductor substance within the ventral ectoderm.

Shen's results cannot be doubted; induction is produced by carcinogens. Shear (1936), Shear and Lorenz (1936), Shear (1937), and Cook, Haselwood, Hewett, Hieger, Kennaway, and Mayneord (1937) also found that induction could be produced by means of carcinogens. However, Shen's statement that the carcinogen induced directly may be questioned. There is a possibility that the inductor was liberated by the carcinogen and that the liberated inductor produced the neural tube formation.

Needham (1942) asserted that the relationship between prepared inductors and natural inductors was very questionable. He stated that synthetic inductors, as described by Shen (1939), might be substances related to the inductor, or that these prepared inductors stimulated the release of the natural inductor. Needham (1942) suggested that if a small dose of carcinogen was required to provoke neural tube formation, and if a small dose of a natural inductor likewise provoked neural tube formation, the carcinogen and the natural inductor were related. However, if a large dose of carcinogen was needed to produce
the same result as a small dose of natural inductor, the carcinogen and the natural inductor were not related. A large dose of carcinogen caused the release of the natural inductor by damaging the surrounding tissue. This concept that the carcinogen and the natural inductor are related, if each produces neural tube formation by use of a small dose, is disputable. A small dose of potent carcinogen could destroy many cells and evoke an induction, but it need not be related to the natural inductor even though it acts in sufficiently small amounts. The relationship between natural and synthetic inductors is still vague. However, an induction is evoked by the action of carcinogens. Whether this action is a direct or an indirect one is still open to debate.

Some investigators maintain that cellular proliferation without differentiation can occur without the presence of an inductor. They believe that the "induction" is accomplished by viruses. However, normal growth, controlled mitotic division, and differentiation do not take place. Rous (1910) showed, for the first time, that a tumor could be transplanted from one Plymouth Rock chicken to another. He suggested that a cell-free extract of a tumor might yield a growth. The theory that viruses produced growths directly, without liberating or disrupting the inductor, originated from Rous. This theory does not agree with the inductor theory of normal and abnormal growth.

According to the virus theory, it is thought that the viruses multiply in the host since, as the tumor grows, more of the active agent can be obtained from the tumor growth. This agrees with bacteriological notions. However, the work of Mangold and Spemann (1927)
should not be overlooked. They stated that, when the neural plate of the amphibian embryo was induced by the inductor influence of the roof of the archenterone, the newly-formed neural tissue acquired the ability to induce a second neural plate when transplanted into the blastocoele cavity of another embryo. Cell-free extracts of the neural tube could also induce. Therefore, there is a similarity between the transferability of a neural tube induction by a cell-free extract, and the production of a tumor by cell-free extracts. A cell-free extract of a tumor is capable of causing an abnormal growth. This fact is thought to support the virus theory, for it is maintained that the virus is transferred to the host and thus the virus provokes a tumor formation. The fact that a cell-free extract of a neoplasm can produce a tumor has not been confirmed by all investigators. Murphy and Sturm (1941) reported that cell-free extracts of tumors failed to produce tumors. Various carcinogens were used to induce tumors in chickens. Extracts made from these tumors did not produce abnormal growths in other chickens of the same species.

Rous and Kidd (1936) offered an explanation of the virus theory so that it would be compatible with the inductor theory of abnormal growth. They produced hyperplasia in rabbits by treating them with tar for three months. When tar papillomas appeared, Rous and Kidd injected large amounts of a Berkefeld filtrate containing active virus into the areas of the papilloma growths. At the end of the incubation period of the virus, the tumors underwent changes, for they became "beefy, discoid and infiltrative". Thus, a tumor was produced by the
carcinogen, tar, and a cancerous condition was brought on by the virus. Two facts were concluded by Rous and Kidd. First, the virus acted as a secondary invader—it converted a non-malignant tumor into a cancer by disrupting the initial inductor evoked by the tar. Second, the fact that the inductor was upset by the virus and produced a malignancy could not be doubted. The virus produced a release or unmasking of the inductor, with the subsequent induction of growth without differentiation.

These investigators also observed that injections of the virus produced malignant growths even in areas in which there were no observable papillomas. Consequently, they reached a third conclusion, namely, that the virus acted directly to produce a cancer.

It may be said that, although it is maintained by some that viruses cause growth without the presence of an inductor, it is also a valid possibility that viruses liberate the natural inductor. In this liberation, the inductor is disrupted and abnormal cellular division and differentiation result.

Barth (1941) stated that he observed differentiation in ectodermal explants of gastrulae of the amphibian egg in the absence of inductor. He stated that the explants were "healthy" and exhibited no cytolysis. He observed an increased number of neural tube formations when he combined pieces of this explant. He further stated that the manner in which the explants were united was an important factor. Thus, polarity was a factor, for the neural tube was formed from cells of the anterior end of the explant. He asserted that, in some cases,
differentiation occurred in ventral ectoderm in the complete absence of the inductor.

Holtfreter (1945) stated that Barth's observation concerning neuralation without an inductor was incorrect. Holtfreter asserted that the medium in which the explants were placed, acted on the explants. The explants were cytolized. The cytolized cells liberated the inductor from the ectoderm that usually did not release its inductor. Neuralation, as reported by Barth, definitely depended upon the inductor. However, the inductoral action was indirect.

The case reported above, regarding neural tube formation without induction, is attacked on the ground that the inductor is liberated by cytolysis. Since the explants of Barth were cut from the gastrula, the injury produced in certain cells of the ventral ectoderm could also be sufficient to liberate the inductor.

The liberation of the inductor can be accomplished in many diverse ways. In attempting to correlate neoplasia and induction, it is of the utmost importance to bear in mind that many diverse substances can liberate the inductor. If certain substances can disrupt the inductor when it is liberated, abnormalities may result.

**INDUCTORS AND NEOPLASIA**

Inductors are found in the embryos of most animals. In the embryo they promote cell growth in a controlled manner. There is strong experimental and presumptive evidence that the inductor is present in the adult form of most animals, as has been demonstrated earlier in
this thesis. Both natural and experimental abnormalities of the embryo may be explained on the basis of a disruption of the growth controller within the embryo. Adult neoplasms may also be related to a disrupted residual embryonic inductor. Since both adult and embryonic neoplasms possess the same characteristics, there is a possibility that they may be produced by the same agent.

The Escape from the Influence of the Induction Field

At first it may seem that the inductor substance has little to do with neoplasia, since the inductor is concerned with a specific differentiation of structures such as, notochord, limb, or optic cup and may not have any bearing on general growth. However, the inductor is concerned not only with the function of induction, but it also governs the structure and axis of the entire body. An induction field is an area that is concerned with general growth. The main characteristic of an induction field is that all of the cells within its sphere of influence are acted upon to form a complete embryo. Cells that are not in its immediate vicinity are also acted upon indirectly by the induction field. Such cells are stimulated to differentiate by cells previously motivated by the induction field. By such direct and indirect action, a complete embryo is formed. In only one part of the entire induction field is a given organ formed. Other organs are formed in different regions of the induction field. A neoplasm or a cancer may be produced by the proliferation of cells which escape from the influence of the induction field. The escape is only one of form and structure, for a neoplasm is similar to the tissue from which it
originates both histologically and physiologically.

The entire question of the possible relationship between neoplasia and induction depends upon whether the inductor is transmitted from the embryo to the adult and persists in the latter. There is strong presumptive evidence that the induction field does persist in the adult organism. Regeneration, healing of wounds, and normal replacement of cells are adult processes that depend upon an inductor for their proper control. The ability to regenerate may be taken as a measure of the persistence of the inductor within the adult. If a tumor is the result of a cellular escape from the control of the induction field in the adult, those animals that regenerate should show a high incidence of tumor formation. However, the literature on amphibian cancer cites more cases of neoplastic growth for anurans than for urodeles. These data may or may not be thought valid. It could be said that since there is no regeneration in the frog, the induction field is absent, and the appearance of neoplasms is not due to the cellular escape from the control of the induction field.

However, Rose (1942) showed that frogs could be made to regenerate if treated with a solution of sodium chloride. Therefore, the inductor probably is present even in the adult frog in a masked state. In the data of reported cases of neoplasms in urodeles and anurans, as compiled by Lucké and Schlumberger (1949), it was revealed that the anurans developed tumors more frequently than did the urodeles. It must be remembered, however, that the frog is used much more frequently as a laboratory animal than is the newt. Furthermore, it
should be noted that the apparent inability of certain amphibians and other forms to regenerate does not imply that they are devoid of induction fields. And since these animals may possess induction fields, tumors may be produced as a result of the failure of the induction fields to exert a control on certain cells.

Berrill (1943) stated that malignancy of a tumor may be explained as a failure or a weakening on the part of the inductor to exert an influence on cellular activity. However, he asserted that it was unlikely that only one cell escaped from the influence of the inductor, but rather that many cells were involved in this escape. He further stated that malignancy was due to a weakened control rather than to an intrinsic change in the cells. Therefore, the transplantation of tumor cells to normal healthy hosts of the same species or race should fail to perpetuate the malignant growth. He maintained that such grafts would be absorbed by the host.

The assumption by Berrill (1943) that more than one cell is affected agrees well with the notion that there may be an induction field present in the adult, as well as in the embryo. The control of tumors transplanted to hosts of the same species may be accomplished by placing the tumors in undisrupted induction fields of a host. These fields exert a controlling influence on the grafts. Therefore, resorption occurs.

Witschi (1930) showed that in Rana temporaria eggs, which were not fertilized immediately after ovulation, abnormalities developed. He waited three days before fertilizing the eggs. He reported that
there was a tendency for axial duplications and accessory appendages to form. He also stated that the most conspicuous pathological feature was the loss of the power of differentiation in the embryonic cells and the tendency of the embryonic cells to develop into neoplastic growths. The loss of differentiating power affected all three germ layers. Neoplasms developed in all three germ layers. The physical alteration of the eggs interfered with the control of differentiation within the egg. Masses of undifferentiated or poorly differentiated cells were formed. In some cases these cell masses were malignant. Thus, a disruption of the inductor field by a physical process in the developing *Rana temporaria* egg produced a neoplasm.

Briggs (1941) studied the effects of delayed fertilization on *Rana pipiens* eggs. He observed abnormalities in gastrulation, such as incomplete gastrulation, exogastrulation, complete closure of the blastopore with deficiencies in the axial structures, partial twinning, secondary embryos, and accessory appendages. He also reported abnormal tissue growth following delayed fertilization. Three types of ectoderm growths were noted as well as growths of the entoderm and mesoderm. He stated that there was only one case of a papilloma and three questionable tumors of the somatic mesoderm. The tumors grew because the cells of the host had a retarded mitotic rate. Briggs did not experiment with the same species of frog egg as did Witschi. This may account for the few malignant growths reported by Briggs. However, the fact that the delayed fertilization affected the inductor within the developing egg cannot be disputed, since Briggs also ob-
tained abnormal growths by a process that interfered with the normal action of the inductor.

Blandau and Young (1939) studied the effects of delayed fertilization on the guinea pig ovum. They reported that delayed fertilization produced abnormalities that were most pronounced during the early stages of development. Therefore, in the mammal, as well as in the amphibian, a disruption of the normally functioning inductor within the developing egg by the physical process of delayed fertilization produced abnormalities. Needham (1936) speculated that overripeness of the egg led to an upset of sterol metabolism. This upset in metabolism led to a deviation in sex hormones, which upset the sex ratio. The disorganized sterol metabolism also produced an uncontrolled liberation of the embryonic inductor, which resulted in the formation of monsters. The metabolism was affected. The inductor was influenced. It did not exert its complete influence on growth and hence, certain cells were able to escape from the induction field.

Berrell (1943) stated that, in his opinion, tumors of a "multiple nature" developed in overripe eggs. He asserted that these tumors were caused by a true weakening of the embryonic induction field and by a general reduction in the rate of proliferation. He also noted that the tumors were the product of certain cells that continued to divide at the normal rate, while the cells in adjacent tissue proliferated at a slower rate than was normal for these cells. In other words, the cells in the area of the "tumor" were actually multiplying at a higher rate than those in adjacent tissue, and thus tumors were
formed. Briggs (1941) reported that the mitotic index of the tumor cells was normal, while that of the host was retarded.

Histological differentiation generally proceeds in the embryo according to location in the induction field. The position of a cell in the embryo influences its development. The cell is directed along a definite path by the induction field. Berrill (1935) stated that tissues that were removed from the induction field of the adult and cultured in vitro exhibited a loss of differentiation, but they showed an increased rate of proliferation. He also stated that the failure to differentiate occurred as a result of the removal of the tissue from the controlling field. He further stated that increased mitotic division also inhibited cellular differentiation. He called the failure to differentiate a dedifferentiation.

Bizzozero and Vassale (1887) classified tissues or cells on the basis of their ability to differentiate. They classified cells as "permanent, stable, or labile". They found that most cells were in the labile class. They stated that nerve and muscle cells were examples of the permanent class, for they were highly differentiated and normally did not multiply. Liver and pancreas belonged to the stable class, since they showed a moderate degree of differentiation and some capacity for cell division. Labile cells were those that showed a low degree of differentiation and a great capacity for division. Fibroblasts belonged in this class. This classification suggests that there is some correlation between mitotic cell division and the degree of differentiation of a cell. It also follows that the more
highly specialized or differentiated a cell, the less is its ability to divide mitotically.

Berrill (1935) stated that the evidence from tissue cultures and fission among Protozoa helped to support the theory that a high degree of differentiation and a low degree of mitotic division were related. Under conditions of tissue culture, many cells divided that did not do so when they remained in vivo. Therefore, it was evident that certain types of differentiation were compatible with mitotic activity, while other types were incompatible. Fischer (1922) and Rienhoff (1922) showed that the epithelial nature of a tissue was maintained whether it was ectodermal, kidney epithelium, or intestinal epithelium. Fischer cultured his cells under ordinary in-vitro conditions, while Rienhoff cultured adult kidney in chick embryos. Lewis and Lewis (1917) reported that muscle fibers retained their power of contraction, although they lost their cross striations when cultured in vitro. While the cultured tissue cells mentioned above actually dedifferentiated when they multiplied, they maintained their epithelial structure and exhibited muscular fibrillation.

Thus, cell division takes place as a result of the dedifferentiation that has occurred or the reduction in the specialized state that has taken place. Therefore, the experimenters mentioned above furnish evidence that highly differentiated cells or tissues do not divide, and that if they are motivated to do so by growing them in tissue cultures, they show a tendency to exhibit a dedifferentiation in the resulting cells. These phenomena may be related to malignancy,
for a dedifferentiation occurs during the proliferation of a cancerous growth. Normally the inductor does not produce chaotic growth, and uncontrolled cell division does not occur. However, the disruption of the normal functions of the inductor by a carcinogen, by some other exogenic substance, or by an endogenic substance permits cell division to occur without differentiation or with reduced differentiation, resulting in a mass of cells that, although dedifferentiated, may still function.

In Protozoa, also, there is a dedifferentiation of the more specialized organelles, flagella, or cilia during fission. When flagellates divide, there is an absorption or dedifferentiation of the flagella, and the resulting daughter cells grow their own flagella. Thus, cell division in these one-celled animals is also incompatible with high differentiation. There must be a dedifferentiation to accompany cell division.

Thus, the experiments performed on tissue cultures and fission in Protozoa furnish evidence for the theory that the nucleus cannot control differentiation during cell growth. There is a resulting dedifferentiation. Therefore, highly differentiated cells do not divide, for abnormalities would result. There is a lack of control.

Berrill (1943) stated that neoplastic tissues frequently were described as dedifferentiated or as having reverted to an embryonic state. The evidence from experiments with tissue cultures supports this statement. Tumor cells exhibited a high degree of cell division that prevented the full expression of cell character. The resulting
cells appeared dedifferentiated or embryonic. The ontogeny, morphology, and, according to Schrek (1936), the growth rate of neoplasms were comparable with those of embryos.

Nicholson (1931) stated that embryonic tumors are not congenital new growths but developmental malformations. They showed the effects of abnormal stimulation during the growth period. Embryonic tumors differed from the developing organs on which they were found. In the case of the tumor cells, growth continued and differentiation was retarded. However, in the case of the organs, both cell division and differentiation were controlled so that a systematic and regulated structure resulted. Thus, as conceived by Nicholson, a tumor was formed as a result of cellular escape from the induction field, and such a tumor manifested itself by a separation of the developmental processes of cellular division and differentiation.

The Separation of the Developmental Processes

The normal function of the inductor in the embryo is to produce regulated cell division and differentiation so that an orderly embryo will result. Inductors, which are probably present in the adult, also function to produce systematic mitotic division and coordinated differentiation so that a cell that is anatomically and physiologically integrated will be produced. If, however, cell division proceeds in either the adult or the embryo at a normal rate and is accompanied by a retarded rate of differentiation, an irregular cell or embryonic growth will be formed. Thus, a separation of cell division from differentiation results in the production of abnormalities. Experi-
mentally, many investigators confirmed the theory that alteration or separation of the developmental processes produces a malformed entity.

Broca (1862) reported that a delay in the incubation period of chickens' eggs resulted in a dissociation of cell growth and organization. Edwards (1902) found that he could influence the normal growth of Anidian blastoderms by altering the incubation temperature. Hoadley (1938) reported that *Rana pipiens* eggs developed abnormalities when they were exposed to supramaximum temperatures. Olson (1942) also reported changes in the body proportions of frogs when the developing eggs were exposed to high temperatures. Mitschi (1930) and Briggs (1941) reported that delayed fertilization of amphibian eggs resulted in the malformations that were characterized by cellular proliferation but retarded differentiation. Piper (1933) reported that *Rana temporaria* developed malformations and abnormal gastrulations when exposed to a solution of sucrose. Dawson (1938) reported that 2, 4-dinitrophenol caused abnormal gastrulations and abnormal growths in developing frogs' eggs. Baldwin (1915) reported that spina-bifida and tumorous growths were produced in developing frogs' eggs. He produced these effects by the action of ultra-violet rays upon the developing eggs. Thus, many factors alter the inductor and cause a separation of cell division and differentiation. The normal relationship between growth and differentiation may be upset.

Hoadley (1930) found that when presumptive embryonic organs of a chick were transplanted into the chorio-allantoic membrane of
another chick embryo, and allowed to reach a point of differentiation equal to that of untransplanted controls, wax models of the structures that developed in the controls were much heavier than those of similar structures that were formed as a result of the transplanting of the presumptive embryonic organs. He further found that a direct relationship existed between the age of the transplant and the size of the organ that was eventually differentiated. For example, the younger the transplant, the smaller was the resulting organ. The chorio-allantoic membrane had an excellent source of food, for it was very heavily vascularized. Therefore, the small size of the transplant was not due to a deprivation of food. It should also be noted that there was a period of lag in the growth of the transplant similar to that which takes place when bacteria are transplanted to fresh agar. This lag in growth affected cellular division and not differentiation. It also affected small cells more than it did large cells. Therefore, Headley concluded that "typical development is the result of the usual balance between morphogenetic (form producing) and histogenic (cell differentiating) processes". Furthermore, abnormal development would be a separation of "morphogenetic" and "histogenetic" processes.

Waddington (1932) and Landauer (1932) presented more evidence to verify the fact that the separation of cell division and differentiation led to experimental dwarfism. Loeb (1892) showed that it was possible to have nuclear division without cell division. He found that a condition of multinuclear, single-celled blastomeres resulted
when sea urchin eggs were treated with hypertonic sea water. Loeb's findings were confirmed by Norman (1896), Morgan (1899), and Driesch (1899). However, these investigators obtained the multinucleated, undifferentiated condition by using magnesium chloride, strychnine, and abnormal temperatures, respectively. It should be noted, however, that a syncytium is found under normal conditions in the phylum Arthropoda in such classes as Hexopoda, Araneida, and Crustacea.

Lillie (1906) reported another unusual phenomenon that further illustrated that developmental processes could be disengaged. He placed unfertilized Chaetopterus eggs in sea water containing high concentrations of potassium chloride and left them in the water for long periods of time. These eggs proceeded to differentiate in the absence of either cell division or nuclear division. He also reported that differentiation occurred without cell division when fertilized Chaetopterus eggs were exposed to high temperatures.

Normally, the inductor effects nuclear division, which affects cell division and growth. The resulting daughter cells are acted upon by the inductor so that subsequent growth results in differentiation. The daughter cells are new with respect to time. However, certain traits of these cells are old in relation to their predecessors. Early cleavage, immediately following fertilization of an egg, is not accompanied by cellular differentiation. As cleavage continues and new cells that possess old traits are formed, the inductor acts on these "aged cells" to evoke differentiation as well as proliferation. Thus, the inductor calls forth differentiation only after cells have aged to a state of competency. The abnormal treatment by Lillie
aged the cells so that they differentiated without cell division, for the condition of the cells and nuclei was at such a point that they were definitely receptive to the inductor, but they were incited to differentiate rather than to divide.

Other experiments illustrate that the developmental processes can be separated, and still, the abnormal growth may be physiologically active. Malignant cells, which are undifferentiated and which are proliferating rapidly, have been reported by some investigators to be functionally stable. It appears that the disrupted inductor has not completely upset the functional capacity of the cellular mass.

Graver and Robinson (1932) reported that active lactation was observed in adenoma of the breast. Lactation was also observed in pieces of the adenoma transplanted into regions not related to the breast. Strong and Smith (1936) found that transplanted hepatoma of C. B. A. strains of mice continued to secrete bile. They observed bright-yellow pigmented cells after the hepatoma was transplanted to regions unrelated to the liver. The fact that cell growth continued and differentiation failed probably accounted for the abnormal formation. However, the disrupted growth process involved only a separation of mitotic division and histogenesis. The tumor maintained its physiological stability.

Black, Kleiner, and Bolker (1949) reported a therapy in human malignant neoplasia that may be related to an inductor and a separation of developmental processes. They inhibited some malignant growths by using fluoride, iodoacetate, and malonate, all of which inhibited
glycolysis. They inhibited tumors in cases of acute leukemia, Hodgkin's disease, and lymphosarcoma. Some responses of a less definitive nature were elicited in cancers of the breast, adrenal cortex, cervix, lung, stomach, and testis. However, they were unable to treat successfully patients with carcinoma of the colon, fundus uterus, ovary, pancreas, and rectum, and squamous cell cancers of the cheek and pharynx. They concluded that certain types of cancer required glycogen for metabolism and that growth ceased when the glycolytic mechanism was removed.

Another explanation of the findings of Black and others (1949) is that the inductors, which may be glycogen-like, are inhibited, and in those cases where they are not inhibited, stronger inhibitors are needed. The inhibition of metabolism, which either inhibits the production of the inductor or interferes with its action, prevents further abnormal growth. New conditions of equilibrium are established. The process of cell division is no longer separated from the process of cell differentiation.

Altschul and Friesen (1949) reported that the volume of the nuclei in tumors was often greater than that which was found in the normal parent tissue. In fact, an individual tumor cell contained a larger nuclear mass than did a single normal cell of the host tissue. The nuclei increased in both size and number. This could be due to a separation of nuclear division from cell division and the subsequent separation of proliferation from differentiation. Thus, an abnormal cell mass or a malignant growth could be produced in this case by an increase in nuclear division without differentiation.
It may be concluded that tumors from both the adult and the embryo possess certain attributes that would lead one to believe that the growth processes are not controlled. In other words, mitotic division and histogenesis are separated. In the embryo it is thought that a disrupted inductor produces the separation that is manifested by an abnormality. In the adult there is good presumptive evidence that a similar situation prevails.

Studies have been made of the growth of tumors, the relationship of such growth to the embryo, and the separation of developmental processes in the embryo. Briggs and Berrill (1941) reported that the ectodermal papillomas produced by delayed fertilization of Rana pipiens eggs were not transplantable. The papillomas were grafted into the anterior eye chamber and into various subcutaneous sites of embryos in the tail bud stage, the cell growth rate of which was normal. They stated that the growth rate of the tumor was normal but that the rate of growth of the original host had been retarded. Therefore, they concluded that a transplant became incorporated into the normal ectoderm of the new host after it was transplanted, for both the tumor and the new host had the same growth rate. In Rana palustris they noted that there was an occasional development of papilloma in the host tissue in some cases of homotransplantation and heterotransplantation.

The transplants reverted back to normal under the apparent ability of the host to regulate the factor that brought about the proliferation without any apparent differentiation. In those few cases in which Briggs and Berrill (1941) noted the appearance of papillomas, the host
either failed to regulate the growth of the papillomas, or the transplant itself may have contained a large amount of the inductor in a disrupted state. Thus, the inductor evoked growths in the host. Metabolic changes may have caused a separation of the growth rates in various parts of the embryo and thus resulted in a high degree of cell division and a lack of histogenesis.

As noted earlier in this thesis, regeneration is an adult growth process. It is probable that regeneration is governed by residual embryonic inductors. Dedifferentiation occurs in the regenerative process. Dedifferentiation is a regression of cell type to a lower state. Huxley (1921) stated that the cells, in their dedifferentiated condition, closely resembled embryonic cells, since both cell types were undifferentiated. Furthermore, another similarity was shown between dedifferentiated cells and embryonic cells by the results of transplantation experiments, for both dedifferentiated and embryonic cells exhibited totipotency when transplanted. Before gastrulation, cells of the amphibian egg exhibited totipotency when transplanted into any region of the blastula. Transplanted dedifferentiated cells from regenerating amphibian limb, likewise demonstrated totipotency when transplanted into the amphibian gastrula.

It is possible to separate the developmental processes in regeneration, for dedifferentiation can be separated from degrowth (reduction in cell size). Wilson and Penny (1930) demonstrated that dedifferentiation existed without degrowth. The morphology of a sponge was disrupted by passing it through a sieve of gauze. The sponge was made
to dedifferentiate by this process, and yet the cell size of the remaining cells was not reduced. In tumors, a comparable situation existed, in that there was no degrowth. However, dedifferentiation took place. In other words, there was a cellular proliferation into an unorganized mass, since a growth of undifferentiated cells resulted.

The fact that dedifferentiation can exist, separated from cell degrowth, cannot be doubted. However, this fact was demonstrated in an animal of low evolutionary significance and under unusual experimental conditions. It is possible that a separation of these two processes, without the necessary inductor to cause differentiation, may lead to a growth of undifferentiated cells. Thus, the separation or escape of certain cells from the influence of the inductor, in developmental and regenerative processes, may almost certainly result in abnormalities.

Teratoma and Its Relation to Induction

A teratoma may be defined as an abnormal growth that contains more than one cell type and that is related to more than one of the three embryonic germ layers. Teratomas are commonly found in the embryo. They are also found in the adult. Many investigators believe that they are produced by a disruption of the inductor within the embryo or the adult. Therefore, a study of teratomas may help to demonstrate a possible relationship between neoplasia and induction.

A teratoma has little organization. It has many tissue parts, but they are not organized into a distinct entity. A teratoma exhibits cellular proliferation of several cell types, but it does not
exhibit differentiation or organized growth. Therefore, a teratoma demonstrates characteristics, which may be caused by a disrupted inductor within the organism.

Teratomas generally originate from gonadal tissue. However, they have been shown to originate from other regions. Barron (1916) reported a case of teratoma of the brain which involved striated muscle cells and goblet cells. Pusch and Nelson (1935) described a congenital teratoma of the thyroid gland that contained components of all three germ layers. Geschickter (1935) described a teratoma of the suprarenals which likewise involved several germ layers. Needham (1936) compared teratoma to the chaotic condition produced when an induction field was upset at some point and the inductor substance failed to exert an influence.

Falin (1940) believed that necrohormones and trephones were liberated during cellular destruction at the site of experimentally produced teratoma of the rooster testis. These substances acted as the inductor, or liberated the inductor, which then acted on pluripotential testicular gonocytes to produce the teratoma. Falin noted that a pluripotential tumor anlage, capable of cellular growth and differentiation, had to be present in the adult testis to produce a teratoma.

Falin (1940) accounted for experimental and spontaneous teratoma of the testis and teratoma of other regions. Teratoma could develop from the primary genital cells or gonocytes. Gonocytes appeared in the chick embryo at a very early stage of development, before the formation of anlagen in the genital glands. The gonocytes were first
concentrated in the region of the germinal crescent. From this location they spread throughout the embryo and finally entered the testis anlage. Falin asserted that it was likely that some of these gonocytes failed to reach their destination and stopped elsewhere. The gonocytes formed genital cells, and it was their presence in the testis of the adult that gave rise to both experimental and spontaneous teratomas.

Thus, this theory explained the presence of pluripotential tumor anlagen in the testis. This theory also accounted for the predominance of teratoma testis and the production of teratoma in other regions. The fact that only ten or eleven percent of the roosters injected with a solution of zinc sulphate developed teratomas, led Falin to the further conclusion that gonocytes perish with age.

If Falin's theory of the origin of pluripotential cells is correct, the appearance of teratoma in regions other than the testis can be explained. The inductor of the adult could act on these pluripotential cells to produce the teratoma. However, the inductor does not regulate the growth of these multipotential cells completely, for a completely organized embryo does not result.

There is another theory that has been proposed to explain the origin of teratoma of the testis. Parthenogenesis has been used by many investigators to explain the phenomenon of teratoma of the gonads. This theory of parthenogenesis has arisen from the experimental work of Loeb (1899). He demonstrated that the sea urchin eggs exhibited nuclear division without segmentation of the protoplasm when these
eggs were treated with sea water to which certain salts had been added. No fertilization was necessary. He suggested that even mammalian eggs might develop parthenogenetically if the concentration of certain ions in the blood were to be altered.

Michalowsky (1932) stated that teratoma was due to parthenogenecity of both the egg and sperm. Teratomas developed when either oocytes or spermatocytes began to cleave and form growths. Edwards and Hawkins (1941) also stated that teratoma might result from a spontaneous development of ova or sperm. As evidence, they cited the fact that the gonads were the most common location for tumors of a teratoid nature.

Edwards and Hawkins (1941) offered another possible explanation for teratoid tumors. They asserted that a teratoma might be formed in the adult as a result of a residual embryonic blastomere that had been isolated in the embryo, was transmitted into the body of the adult, and there developed into a teratoma of the adult. Segregation of such a relatively undifferentiated cell or cell group, temporarily inhibited in development, would have the necessary potentialities (ability to yield three germ layers and many cell types) to form a teratoma, once the blastomere became reactivated by an inductor substance. Marchand (1897), Schwalbe (1907), and Greil (1924) likewise agreed that the inclusion of isolated blastomeres, when stimulated to grow, could exhibit various degrees of differentiation manifested by equal conjoined twins, or irregular conjoined twins, or teratomas.

Falin (1940) stated that there was no evidence for the partheno-
genetic theory of the origin of teratoma. It is rather difficult to conceive of oocytes undergoing cleavage in vivo without stimulation. It is even more difficult to concede that spermatocytes can develop parthenogenetically. Furthermore, the presence of teratomas in regions other than the gonads cannot be explained satisfactorily by the parthenogenetic theory.

Two types of cells have the capacity to form teratomas. They are mature ova and totipotent cells that remain in various sites in the body of the adult. Mature ova are specialized cells and, under proper stimulation, they can undergo proliferation and differentiation to form an embryo. Immature ova lack these capacities and cannot develop when they are stimulated. Sperm cells are not capable of developing into an embryo when stimulated. Therefore, it does not seem very likely that parthenogenetic cleavage of sperm cells could produce a teratoma in the testis. Therefore, since only mature ova are capable of developing under either normal or parthenogenetic stimulation, it does not seem likely that the theory of parthenogenecity can explain teratoma in any site other than the ovary.

It is more likely that inductors within the adult or embryo act upon pluripotential cells in the adult or embryonic body and thus start a growth. The growth that is produced is not a complete animal but rather a mass that results from an attempt by the host to produce another embryo. The end product resembles a tumor, since it shows a proliferation, but it does not differentiate into any distinct form. It is probable that the inductor may be disrupted or that it is not as
potent in the adult condition as it is in the embryo. Therefore, a teratoma results. However, there is a possibility that the pluripotential cell is not in the same physiological state as embryonic tissue and hence, a teratoma results. Moreover, if the inductor is not disrupted, and the pluripotential cell is competent, there is a possibility that a second embryo could result in a host embryo or in an adult form. Restrictions imposed upon pluripotential cells by either the embryonic body or the adult body would interfere with growth, and teratomas would result.

Falin (1940) suggested a possible origin for pluripotential cells. There are other evidences that indicate that cells capable of further growth are present in the adult. Such unspecialized cells exist in most invertebrates and vertebrates. The mesenchymal cells of Porifera and Coelenterata, for example, are unspecialized cells. The fibroblasts and mesenchyme cells of vertebrate connective tissues are other examples of pluripotential cells. (This has been shown by tissue culture experiments). Pluripotential cells are responsible for regenerative capacity. The degree of regenerative capacity depends upon the degree of dispersion of these unspecialized cells as well as upon the ability of the inductor to act upon the totipotent cells. Unspecialized cells are found throughout most vertebrate tissues. Histological examination reveals this fact.

Under certain circumstances pluripotential cells are capable of not only regenerative development, as in certain amphibia, but they are also capable of total development, as in tunicates. Thus, since
pluripotential cells are present, and since they have further developmental capacities, the inductor that is probably present may act upon these cells to produce a teratoma.

Although the possibility that teratomas may result from parthenogenesis of oogonia cannot be excluded, the occurrence of teratomas in other regions, as well as in the gonads, can also be explained by the presence of pluripotential cells and inductors.

There is another possible origin for teratomas. Edwards and Hawkins (1941) stated that certain blastomeres or embryonic cells may remain in the adult and then develop at a later time. Since every cell of the embryo is thought to contain the inductor, the residual embryonic cells may also contain the inductor. These embryonic cells develop in the host as undifferentiated cells. It is probable that they are under the control or regulation of the host inductor. Since these cells have a latent developmental capacity, they may develop at any time if they escape from the control of the host. In these cells, the inductor, which is masked by the host inductor, may exert an influence on cell control when it is freed from the host control. The embryonic cells may proliferate and differentiate, and they may form a tumor or teratoma, for the inductor within these cells may well be disrupted or altered by an ageing process within the host.

Briggs (1941) demonstrated that teratomas developed in *Rana pipiens* eggs following delayed fertilization. He reported that partial twinning, secondary embryos, and accessory appendages formed in as many as twenty percent of the fertilized overripe eggs. He also
asserted that these teratomas were formed as a result of the disruption of the inductor within the egg and that they were produced by the process of delayed fertilization. Thus, there was a definite possibility that a disruption of the inductor could produce teratomas.

The teratomas may or may not be considered as tumors. Briggs (1941) reported another observation on those embryos that showed partial twinning, secondary embryos, and accessory appendages. These embryos did not possess tumor-like growths. However, partial twinning, secondary embryos, and accessory appendages may be teratomas. Since a teratoma is a type of tumor, the appearance of the abnormalities such as partial twinning, may demonstrate a tumor formation. Thus, different types of tumors are produced on embryos by the experimental disruption of embryonic inductors. Jackson and Brues (1941) stated that a growth that involved more than one cell type was a tumor. They also stated that, in all probability, tumors were produced by a disruption of the inductor. They further stated that a tumor of the ovary of a mouse assumed biochemical characteristics, such as high glycolysis. These characteristics were common to most true tumors.

Thus, experimental studies of teratomas indicate that a relationship may exist between induction and neoplasia.

**THE RELATIONSHIP AND INTERRELATIONSHIP OF CARCINOGENS, HORMONES, AND INDUCTORS**

There is a definite relationship between the inductor, certain carcinogens, and estrogens. All three produce growth. The inductor normally produces cellular division and differentiation. Carcinogens
promote abnormal cell division and histogenesis. Estrogens cause periodic changes in certain tissues and stimulate the hypertrophy and differentiation of such tissues. There is a similarity between the mechanisms by means of which these three substances act. Also, the chemical structures of certain ones of these substances are similar. And, likewise, the dosages of these substances required to produce their effects are similar.

As noted earlier in this thesis, many diverse agents may be capable of causing a normal induction in embryonic tissue. Likewise, one of the complexities of neoplasia is the large number of diverse agents that is capable of producing abnormal cell growth. Radium, x-ray, ultra-violet light, polycyclic hydrocarbons, sex hormones, arsenic, viruses, and tumor extracts are some of the substances that have been used to produce neoplasia. Since these substances produce the same effect, they can all act the same way, or they can act independently and have the same result. Perhaps these various agents disrupt the inductor, allow certain cells to escape from the induction field, and thus result in an abnormality. There is also the possibility that the various agents used to produce neoplasia act directly on the cells, alter them in some manner (through the chromosomes), and hence produce neoplastic growth. This thesis is concerned with the mode of action of carcinogenic and estrogenic agents on the inductor, and the effects which these agents may have on the inductor.

Waddington (1938) reported that certain carcinogens and estro-
gens in albumin suspension exhibited inductorial power when they were implanted into the blastocoel of *Triton alpestris* gastrulae. Controls of albumin suspension showed no induction. Therefore, he concluded that the induction by synthetic carcinogenic substances may be due to the implanted substances themselves, for they may have been similar to the inductor, or they may have liberated the natural inductor, in a disrupted form, by injuring the cells of the gastrulae.

The relationship between certain hormones and certain carcinogens can be demonstrated. It is of significance that some hormones, which regulate certain physiological processes, can be converted chemically into carcinogens. Assuming that Waddington (1938) is correct, then the converted hormones, acting as carcinogens, may induce malignancy.

Corticosterone, one of the hormones secreted by the adrenal cortex, and progesterone, the hormone that governs the secretory phase of the menstrual cycle of the human female, have a very similar chemical structure. It is possible that they can be altered chemically and that they can be interchanged. Needham (1942) stated that if the side chain of progesterone were to be cut down, testosterone would be obtained. Further aromatization would yield oestrogen and, if ring II also were to be aromatized, equilenin would be produced. Aromatization of equilenin would lead to the formation of a carcinogenic substance, methylcholanthrene. Thus, it was shown that it is possible to pass from a hormone to a carcinogen by first going through a sex hormone stage.

Cook, Dodds, Hewett, and Lawson (1934) demonstrated that there
was some relationship between the stimulus for cancer production and the stimuli of the sexual cycle. They injected approximately one tenth of a gram of 5:6-cyclo pento - 1:2 benzanthracene into some rats of the Wistar stock and one tenth of a gram of 1:2 benzpyrene into other rats of the same stock. (One hundred milligrams of each substance were dissolved in three cubic centimeters of sesame oil. One cubic centimeter of solution was injected on each of three successive mornings in different parts of the animal's body). They found that 5:6-cyclo pento - 1:2 benzanthracene and 1:2 benzpyrene were estrogenic. Barry, Cook, Haslewood, Hewett, Hieger and Kennaway (1935) found that these substances were carcinogenic. The difference in effect reported by these two groups of experimenters may be attributed to the different dosages that they used. Cook and others (1934) also found that 9:10-dihydroxy-9:10-di-n-butyl-9:10 dihydro-1:2:5:6-dibenzantracene and 1:9-dimethyl-phenanthrene were estrogenic. Waddington and Needham (1935) reported that these substances evoked an induction. Cook and others (1934) further stated that chrysene and 1:2:5:6:-di- benzanthracene did not produce estrogenic, carcinogenic, or inductoral effects at the dosage used. They concluded that there was a whole group of substances that varied greatly in structure and that could produce estrogenic effects.

Waddington and Needham (1935) tested various synthetic polycyclic hydrocarbons by implanting them into the cavities of young amphibian gastrulae. The test substances were implanted after they had been emulsified and coagulated into small pieces by mixing them with
egg albumen. The concentration of the substances used was about two milligrams per cubic centimeter. They reported that induction could be accomplished by the use of 1:9-dimethylphenanthrene, 9:10-dihydroxy-9:10-di-n-butyl-9:10 dihydro-1:2:5:6-dibenzanthracene, and 1:2:5:6-dibenzanthracene. The evidence of induction was clear when the first two substances were used. However, induction was not definitely shown for 1:2:5:6-dibenzanthracene, since this substance produced only one neural tube induction in Discoglossus and only minor effects in newt embryos. Certain synthetic hydrocarbons of the phenanthrene group possessed a capacity to induce. The first of the substances used was known to be estrogenic and the third substance was known to be carcinogenic. There probably was a group of phenanthrene substances which could act as inductors, some of which were estrogenic, while others were carcinogenic.

Barry, Cook, Haslewood, Hewett, Hieger, and Kennaway (1935) used many of the compounds which had been used by Waddington and Needham (1935) and Cook, Dodds, Hewett, and Lawson (1934), but they reported different results. Barry and others (1935) tested the carcinogenic activity of various substances by applying them, in solutions of benzene, to the interscapular region of mice twice daily. (Three-tenths of a gram of substance was dissolved in one hundred cubic centimeters of benzene). Tests for cancer production were performed with polycyclic aromatic hydrocarbons or closely related compounds (tetracyclic or pentacyclic compounds). They reported that the active compounds were derivatives of 1:2 benzanthracene. The two derivatives of 1:2
benzanthracene that had considerable cancer-producing power were 1:2-benzpyrene, a compound isolated from coal tar pitch, and methylcholan-threne, a compound derived from the desoxycholic acid of bile.

Since the three groups of investigators mentioned above used the same chemical substances in certain experiments, and since they obtained different results, there must be some factor that was not similar to the three groups. The dosages used by the different investigators varied. At a given concentration a substance induced. At another concentration it acted as a carcinogen. At still another concentration it had the effect of an estrogen. Consequently, a variation in dosage produced diverse results.

The interrelationships that exist between the actions of the compounds tested are not due to any chemical similarities between them but rather to the mode of action that they all exert on the target areas that they stimulate. At different concentrations the substances can induce, cause malignancy, or produce estrous. In induction there is a controlled liberation of substance that produces normal growth. In malignancy an induction is produced, but the substance released is probably not the same as that which is released under normal growth, and the altered substance evokes a proliferation but not a differentiation. When chemical substances produce estrogenic effects, such as take place in the growth of the vagina, uterus, ovary, and other secondary sex structures, there is a need for controlled growth after these organs are stimulated by hormones or synthetic hormones in order to start a proliferation. The proliferation, which is evoked in this
manner, must be regulated, or there will be a tendency for a malignancy to develop.

The investigators mentioned above have shown further that not only do 1:2:5:6-dibenzanthracene, 5:6-cyclopenteno-1:2-benzanthracene, chrysene, methylcholanthrene, and 1:2-benzpyrene have different effects (inductor, carcinogenic, or estrogenic) depending upon the dosage, but they even have different effects with the same dosage. They probably have different abilities to liberate the inductor, assuming of course that the target area is always the same.

From the work cited above, a possible relationship may be assumed between the inductor, certain carcinogens, and certain sex hormones. The work of Lathrop and Loeb (1916) indicated that there was good experimental evidence for this assumption. They castrated female mice (strains of English Sable, English Mice, and English 101) that were less than six months old and reported that there was a marked decrease in the incidence of cancer in the mammary glands of these animals. However, cancer was not prevented. These castrated female mice developed cancer at an older age than did non-castrated mice. Castration of those mice that were older than six months did not affect the cancer incidence. Lathrop and Loeb stated that the corpus luteum influenced the development of cancer in the mammary glands.

Castration of female mice removes the source of the estrogen hormone. Since estrogens can be converted into carcinogens, a possible carcinogenic agent is removed. The agent that induces the mammary glands to proliferate and differentiate is also removed.
incidence of cancer is decreased, the agent that may alter the induc-
tor may be removed by castration. The incidence of proliferation
without differentiation in the mammary glands is thereby reduced.
There is also the possibility that if the inductor is sterol-like, the
estrogens, which are also sterol-like, may stimulate the neoplastic
growth directly. Since it is probable that the estrogens are not ex-
actly like the natural inductor, they may promote an abnormal growth.
The results of the experimental work of Lathrop and Loeb (1916) defi-
nitely increase the possibility of a relationship between the inductor,
estrogens, and carcinogens.

Cori (1927) reported results that further confirmed the above
theory of interrelationship. He reported that castration of female
mice (strain 3 of the State Institute for the Study of Malignant
Disease, Buffalo) between fifteen and twenty-two days old entirely
prevented the occurrence of spontaneous adenocarcinoma of the breast.
Non-breeding control mice of the same strain, which were not castrat-
ed, showed a tumor incidence of seventy-nine percent. Castration of
mice that were between two and six months old led to a marked reduction
in the tumor incidence, but it did not prevent the occurrence of mam-
mary tumors. He concluded that the spontaneous mammary carcinoma of
these mice was due to an hereditary disposition. This disposition re-
mained latent in the absence of ovarian function, but cancer became
manifest when a certain amount of ovarian hormone, an amount corre-
ponding to five to thirty estrous cycles, had acted on the breast tis-
sue.
Lacassagne (1936a) reported that certain estrogenic substances were carcinogenic. He also reported that certain carcinogenic hydrocarbons were estrogenic. He stated that estrone, equiline, and equilinine produced modifications in the mammary glands, uterus, prostate, and hypophysis after continued injection of these substances into mice. Cancer appeared soon after the injection of estrone, but it did not appear as rapidly after the injection of equiline or equilinine. It is of significance that the more closely the estrogen resembled a sterol, the greater was its ability to produce a neoplastic growth. A similarity to a sterol-like inductor is indicated.

Suntzeff, Burns, Moskop, and Loeb (1936) reported that it was possible to increase the incidence of mammary cancer in mice by long continued injections of estrin. The effects varied with the hereditary tendency of a given strain to develop cancer, and the effects also varied with the dosage. They further demonstrated that cancer of the mammary glands could also be produced in male mice of high-tumor strains. They produced tumors in such males almost as readily as in non-breeding females of the same strain. The hormone, which was similar to the sterol-like inductor, evoked a cellular proliferation without any differentiation. Thus, the hormone, if it acted as an inductor, could not evoke the same response as the natural inductor. The hormone may have acted on the inductor and altered it. Certain cells were able to escape from the control of the altered or disrupted inductor. These cells that escaped developed into a malignant growth. The hormone definitely acted as a carcinogen. The possi-
bility that hormones are etiological agents in cancer production should
not be overlooked. The hormones may act directly or indirectly to pro-
duce their effects.

Allen (1942) reported that long continued treatment of experi-
tmental animals with high doses of estrogenic hormone was followed by the
appearance of tumors and cancers in these animals. Genital organs
were involved primarily, but non-genital tissues were also influenced.
Therefore, he concluded that endocrine secretions appeared as impor-
tant factors in the genesis of some atypical growths.

Witschi (1933) showed that there might be some relationship be-
tween inductors, carcinogenic hydrocarbons, and steroid compounds. In
his work with overripe eggs of frogs, he obtained abnormal sex-ratios
and sex-determining mechanisms as well as monsters and malignant
growths. Thus, the liberation or production of the inductor was dis-
turbed. Since sex hormones have a sterol structure, and since the nat-
ural inductor may have a structure that belongs to the sterol group,
and, moreover, since certain carcinogenic agents have a related ring
structure, the results obtained by Witschi may be interpreted to mean
that a disturbance of steroid metabolism or a possible disruption of
the sterol-like inductor took place. Since the sterol metabolism was
disrupted in the overripe egg, there was a disruption of the sex-ratio.
The inductor that was released was not normal, and there was a malig-
nant cell-growth tendency.

Falin (1940), who reported that teratoma of the rooster testis
could be produced by solutions of zinc sulphate, also observed that
the evocation of teratoma occurred only in the Spring of the year. It was possible to correlate neoplasia with induction and sex hormones by Falin's observation. There was little success in obtaining an induction with the male sex hormone andosterone. However, the work of Falin did not preclude this possibility. Andosterone was present in very high concentration during the Spring, and since this was the only period of the year during which teratomas were evoked, the male sex hormone may have functioned to produce the teratomas. The solution of zinc sulphate produced necrosis of testicular tissue. The dead cells may have released andosterone that, in turn, stimulated teratoma formation directly, or the liberated andosterone evoked the release of the inductor, in a disrupted form, that resulted in the formation of a teratoma. Therefore, there was good presumptive evidence that a sex hormone acted as a carcinogen and produced a tumor. The inductor may or may not have been activated by the process.

Lacassagne (1936b) stated that the cancer problem was concerned with the liberation of one cell from a group of cells. He asserted that there were two possible ways in which this liberation could be accomplished, 1) by the loss of something that henceforth rendered the cell incapable of obeying the regulatory processes of proliferation and differentiation, and 2) by the acquisition of something acting as a permanent stimulant. Thus, by so stating the problem, he became concerned with those products that altered cell division. Such products came either from within the cell or from outside the cell. Such exogenous factors as parasites, viruses, radiation, and amino acids
provoked the appearance of many mitoses and cell growths. The endo-
dogenous substances were produced by the organism and controlled
growth. Lacassagne considered the following endogenous substances
as the agents that altered cell division: 1) those substances that
promoted wound healing, 2) the inducers that regulated cell division
and differentiation, and 3) the sex hormones that regulated growth of
certain cells.

The exogenous and endogenous substances mentioned by Lacassagne
(1936b) may liberate the inductor. The liberated inductor may not be
normal. An abnormal inductor, liberated by a carcinogen or an estro-
gen, could evoke abnormal cell growth. A neoplastic growth could re-
sult.

The chemical substance 1:2:5:6-dibenzanthracene and related com-
pounds were utilized by certain investigators mentioned above, since
these compounds were reported to have estrogenic, carcinogenic, and
inductor effects. The dosages used to obtain these diverse results
varied in the case of each effect reported. It cannot be assumed that
estrogens, carcinogens, and the inductor are alike chemically. How-
ever, there is strong presumptive evidence that the substances that
control growth are related functionally. There is also strong evi-
dence to indicate that estrogens can be transformed into carcinogens.
Carcinogens produced in this manner can promote abnormal growth or act
on the inductor present, disrupting it and then producing an abnormal
growth.

The high incidence of uterine cancer may be related to the
constant changes in growth that take place in this structure and to
the high concentration of estrogens. It is possible that the estrogens
that are present in the uterus become carcinogens. The carcinogens
could disrupt cell growth by disrupting the inductor. Thus, tumors
would be produced.

At present the following conclusions may be drawn concerning the
relationship and interrelationship of carcinogens, estrogens, and the
inductor:

(1) Certain carcinogens may evoke induction in the embryo.
(2) Certain estrogens may evoke induction in the embryo.
(3) Certain carcinogens have estrogenic effects.
(4) Certain estrogens have carcinogenic effects.

A more thorough understanding of the relationship and interrelationship
of the inductor, sex hormones, and carcinogens may prove helpful in un-
derstanding how neoplasia originates.

A CONCEPT FOR THE EXPLANATION OF THE RELATIONSHIP BETWEEN NEOPLASIA
AND INDUCTION

A concept that is applied in the field of endocrinology may be ap-
plied to the problem of neoplasia and induction. This concept may be
called the target area concept. In endocrinology the target area is
an area or an organ that is stimulated by an endocrine principle. The
state of the target area must be taken into account when an endocrine
secretion is applied to the area. If the physiological state of the
target area is constantly changing, then the same endocrine principle
can produce a different result, depending upon the state of the target
area at the time that the endocrine principle is applied. Many diverse results can be obtained with the same endocrine principle and the same target area at different times.

In discussing the possible relationship between neoplasia and induction, it is also essential to consider the state of the recipient cells. The normal inductor acting upon cells that are changing continually in physiological state could produce different results. Thus, the same dosage of a polycyclic hydrocarbon may produce inductorial, estrogenic, or carcinogenic effects depending upon the state of the target area.

Teratoma in the adult may be produced by physiological alteration of the pluripotential cells. Normally the pluripotential cells may be unaffected by the inductor. However, it may be that these cells (rather than the inductor) become altered and develop into teratomas after they are stimulated by the normal inductor. The process of ageing could affect pluripotential cells within the adult. They could remain in an inhibited state, that is, in a state that is not receptive to the inductor. However, under the stimulation of certain chemicals or endogenic substances the inhibition could be removed from the pluripotential cells. These aged cells would then be stimulated by the normal inductor and produce tumors that resemble unorganized embryos.

Carcinogens could alter cellular stability without upsetting the inductor. The disrupted cells would not react to inductorial stimulation as normal cells react. The injured cells would produce a neoplasm under the stimulation of the natural inductor. Carcinogens, as
well as other substances that injure cells or alter cellular constitution, could change the target area, and this area could become tumorous if it were stimulated by normal inductor.

The target-area concept can also be applied to non-regenerating animals. The cells of such animals may have been altered so that they are not receptive to the inductor, even though the inductor may be present in these animals. However, the inductor cannot stimulate the cells present in these animals, for these cells may have been altered to such an extent that they cannot be affected by the inductor. However, as shown earlier in this thesis, a strong salt solution, if present in a regenerating area, will evoke regeneration in frogs that normally do not regenerate in the absence of a salt solution. The salt solution may alter the physiological state of the target cells in the regenerating area, and then the normal inductor may evoke regeneration in these cells.

Ryan (1941) stated that different time-temperature relationships accompanied cleavage. He asserted that different inductors were responsible for the differences in temperature that accompanied the early stages of cleavage. He also stated that, in later stages of cleavage and in organ differentiation, the same inductor may be responsible for different temperatures. It was not necessary to speculate on the possibility of many inductors being present. The same inductor stimulating altered blastomeres could produce different time-temperature results. The state of the blastomeres changes constantly during cleavage. These changed blastomeres could respond differently to the
same inductor.

Hall (1942) reported that lithium chloride produced exogastrulation in amphibian eggs that were exposed to this salt. He stated that the exogastrulation resulted in the formation of differentiated organs in the evaginated entomesodermal mass but not in the remaining ectodermal regions. These results may be taken as evidence that the inductor was necessary for neural and other ectodermal differentiation. The target region, in this case the ectoderm, may be so altered by the salt solution that the inductor is not effective in its action. Therefore, it may be concluded from the results of this experiment, and from the results of other experiments cited earlier in this thesis, that the quality of the inductor, as well as the quality and quantity of the receptive or target area, must be considered when explaining the possible relationship between the inductor and neoplasia.

**THE RELATIONSHIP OF REGENERATION, INDUCTION, AND NEOPLASIA**

Regeneration is an example of adult tissue replacement and growth under the possible influence of an inductor. It has been stated earlier in this thesis that regeneration is probably controlled by the inductor that has survived the embryonic state. Regenerating tissue is similar to embryonic tissue. Regenerative development is similar to embryonic development. In regeneration, as in embryonic development, an inductor acts on undifferentiated tissue to produce a new entity.

Barth (1938) reported that the rate of regeneration was affected by the available oxygen supply. The rate of regeneration was increased when the oxygen tension was increased. Embryonic growth is also af-
fects by the oxygen supply. Barth stated that the inductor present in both the embryo and the adult depended on the available oxygen supply for its action. Regenerative development was similar to embryonic development in that both depended upon oxygen tension to activate the inductor that functioned in these processes.

Schotte (1937) demonstrated another similarity between regenerating tissue and embryonic tissue. He stated that both processes were concerned with undifferentiated totipotent cells. The mesenchyme of regenerating limbs of amphibians was totipotent. Typical lenses were obtained by transplanting mesenchyme of regenerating limbs into the eyes of adult amphibians that were previously deprived of their lenses. Schotte (1937) further tested the potency of this mesenchyme by transplanting embryonic eye cups of Rana pipiens embryos under the skin of regenerating tails of large tadpoles. The eye cups not only induced the regenerating mesenchyme to differentiate into typical lenses, but they also induced, in every experiment, a complete redifferentiation of the surrounding cells. The loose mesenchyme became a dense mass of cells. Numerous mitotic figures were formed. Eventually, Schotte observed the differentiation of organs that had no connection with eyes. He observed olfactory capsules, ear vesicles, and mouth cavities that appeared in the midst of the tadpole tail. The effect of embryonic inductors upon regenerating tissue can be explained by the spreading of the inductor. In the induction of regenerates, there was a continuous supply of undifferentiated cells. After certain cells were induced by the inductor, they evoked cellular growth and differen-
tiation in the remainder of the proliferating mesenchyme. These experiments suggested that proliferating mesenchyme in regenerating areas embodied properties of undifferentiation similar to those of the embryonic ectoderm of amphibians before gastrulation.

Transplantation and induction experiments have shown that embryonic cells revealed only a small part of their potencies during normal development. Hence, cells of early amphibian embryos were totipotent within certain limits. The totipotent quality within the developing egg disappeared progressively with age. Gastrula ectoderm exhibited unlimited developmental potencies. Neurula and tail bud stages have revealed marked limitations in their morphogenetic potentialities. Transplantation of still older embryonic tissues showed that cells that were formerly totipotent acquired restricted potencies.

Totipotency appears to be limited to a period of very short duration and reserved to cells of a transitory embryonic stage. After this brief period, the cells of the embryo become set in their fates and no new changes in differentiations may occur. Such cells are "determined." Their fate is irreversible.

Harrison (1937) suggested that the words "determined" and "determination" be dropped from the terminology of embryology, for these words were not helpful in promoting the understanding of the developmental processes. There was no criterion for ascertaining when this "determined" condition was reached, if it ever was reached. Harrison (1933) warned that there was no way of concluding with certainty whether the potential characteristics of a cell were finally estab-
lished, since there might be new conditions not yet tested under which other potencies might be revealed.

Schotté and Hummel (1939) stated that adult newts and anuran tadpoles exhibited "determination" in their cells, since under normal conditions they would not exhibit any other differentiation than that which they already manifested. However, amputation of a leg or a tail led to a series of processes similar to those that characterized embryonic development. At the surface of the amputation, cell proliferation led to the formation of a blastema that showed potentialities of progressive self-differentiation not unlike the potentialities observed in the growing embryo. Milojevic (1924) also stated that the blastema formed during amphibian regeneration was capable of progressive self-differentiation.

The regenerate, like embryonic tissue, goes through a period during which it is unable to differentiate into complex organic structures if the connection with its own stump is disrupted. de Giorgi (1924) demonstrated that young regenerates of the tail of Salamandra transplanted onto the side or back of another newt would not differentiate into tails with axial structures. Moreover, Gurvitsch (1922) and Guyenot (1927) stated that the manner in which the blastema of regeneration differentiated was not dependent upon its inherent potencies but rather upon the organizing action inherent in the induction fields or territories of the blastema's location.

The fate of the regenerate depends upon the location into which it is transplanted. Guyenot and Schotté (1927) reported that this was
true for tail and leg induction fields. They demonstrated that a young tail blastema transplanted into the leg field acquired the structure of a leg, and a leg regenerate became a tail when submitted to the action of the tail field by transplantation.

In amphibians, in which the entire induction field was removed and regeneration was prevented, it was shown that the formation of well-defined structures depends upon the inductorial action of a field. Schotte (1926) found that this was true when he excised a tail of Triton in such a way as to remove the entire field. He obtained no regeneration.

Schotte and Hummel (1939) stated that, in order to differentiate, the regenerate must be controlled by an inductor that would direct and control its development. The similarities between the behaviors of regenerates and embryonic tissues suggested that regenerating tissues were totipotent and that they were endowed with properties similar to those of early embryonic tissues. Therefore, regenerating tissues of the urodele, as well as those of the amuran tadpole, could be capable of forming embryonic organs if such regenerating tissues were properly submitted to the action of induction fields.

Lewis (1904) showed that the eye-lens relationship of the early amphibian embryo exhibited a case of dependent development. Wachs (1914) reported the results of Wolffian regeneration in the amphibian eye. From the results obtained by Wachs (1914), it may be concluded that the eye of the adult amphibian continued to act as an inductor. Transplants into the eye chamber, with its morphogenetic field, showed
that induction in adult tissue was possible. Regenerating tissues of urodeles and anurans are totipotent, in the sense that they are capable of differentiations that are normally observed only in embryonic tissues.

Totipotency has also been shown in regenerating tissue of the chick embryo. Zwilling (1942) reported that the removal of the entire tail from chick embryos having seventeen to twenty-seven somites resulted in the absence of the tail in the adult. If a portion of the undifferentiated tissue of the tail anlage remained, there was restoration of the tail. As in amphibia, removal of the entire tail or leg removed the induction field, and thus there was no regeneration. However, if some induction field remained, it acted on the totipotent cells and produced a new tail or leg. Therefore, it can be concluded that regenerative tissue and regenerative development are similar to embryonic tissue and embryonic development.

Berrill (1935) stated that there was a need for cell division during regeneration. This need was greater during regeneration than during growth. The same correlation that existed in tissue cultures between differentiation and cell division existed in regeneration. Highly differentiated cells might survive, but they did not play a part in the proliferation of new cells. It was the non-specialized cells that differentiated into the new structures.

Wilson and Penny (1930) showed that the collar cells in sponges (Microciona) proliferated and produced new collar cells, but they lost their flagella in the process. They also showed that the remainder of
the animal developed as a result of the division and proliferation of unspecialized mesenchyme cells. Coe (1934) demonstrated that regeneration in Nemertians depended upon reserve mesenchyme cells rather than upon specialized tissue cells. Stone (1933) reported that there were some differences in the unspecialized cells involved in the regeneration of the anterior and posterior ends of the Annelid, Tubifex. However, unspecialized cells were involved in the regeneration of either end. Faulkner (1932) reported that all new tissues in Chaetopterus were formed from neoblasts that were non-specialized cells lying in a double strand between the nerve cords. These cells moved about and proliferated.

In the experiments noted above, it was the embryonic or undifferentiated tissues that developed under proper stimulation. These tissues were probably residual embryonic tissues, and they regenerated under inductorial stimulation. Cells may dedifferentiate during mitosis and redifferentiate after mitosis. Redifferentiation is always along the original lines. Dedifferentiation affects only the structure of a cell. Regeneration is a process that demonstrates that mitotic division and differentiation are inversely related.

Experiments have been performed that related regeneration to induction. Horn (1942) demonstrated that regeneration was prevented in the forelimb of larval Amblystoma by the application of adequate dosages of neutron radiation. The extent of regeneration varied with the dosage of radiation. High dosages prevented the formation of a blastema with the consequent occurrence of much dedifferentiation.
Blastema formation stopped dedifferentiation. The mitotic activity of the blastema cells was suppressed by the radiation. The blastema that was normally formed in a regenerating area stopped dedifferentiation. The blastema was then acted upon by the inductor and was capable of being converted into differentiated structures of the leg. If a blastema did not form, the inductor had nothing to act upon and consequently regeneration failed.

Thornton (1943) reported that the results of colchicine treatment were similar to the results of x-ray treatment, since colchicine prevented blastema formation in Amblystoma. Dedifferentiation was not checked and no regeneration occurred. The colchicine probably inhibited mitotic activity, and it was by this means that it was effective in preventing the blastema formation. Blastema formation is the key to regeneration. It is the blastema that has the potentialities to grow when it is stimulated by the inductor. It might be said that the x-ray and colchicine treatments disrupted the target area and that the inductor was unable to evoke a response in this altered target area.

Emerson (1940) reported that regenerating tissue responded, at least partially, to the action of certain embryonic inductors. He also reported that embryonic tissues exhibited a high power of self-differentiation in the abnormal environment of the blastema. Contrary to the experimental findings of Schotte and Hummel (1939), Emerson (1940) reported that regenerating tissue of *Rana pipiens* or of *Rana clamitans*, when grafted adjacent to the embryonic eye cup or presumptive medulla, formed loose, irregular mesenchymal tissue. The grafts
did not form lens or otic vesicle.

Emerson (1940) further demonstrated that presumptive medulla, spinal cord, and forebrain of the embryo differentiated nearly perfectly in the midst of blastema tissue. A lens was formed from the rim of the embryonic eye cup when the presumptive forebrain was grafted into the blastema. Vesicles, similar to early ear vesicles, were formed from blastema tissue adjacent to the grafted embryonic medulla. There were no structures similar to early ear vesicles next to the grafted presumptive spinal cord or forebrain. It may be concluded that the embryonic medulla, with its induction field, evoked ear vesicle formation in the undifferentiated cells of the blastema. Ears with incomplete cartilage capsules formed when either the otic vesicle or presumptive otic vesicle ectoderm was grafted into the blastema. The presumptive ear vesicle ectoderm was transplanted without mesentodermal material. The cartilage capsules were of blastema origin. Embryonic otic vesicle induced cartilage formation in the blastema. The cells that constituted the blastema were of an embryonic type (totipotent and undifferentiated), since the undifferentiated cells differentiated into appropriate tissue types under the influence of induction fields.

Normally, regenerating tissue is regulated by an inductor that promotes the replacement of lost parts by stimulation of the blastema cells. Neoplasms are growths that proliferate but do not differentiate. Such growths exhibit symptoms of a disrupted inductor. Rose and Wallingford (1948) reported that unorganized growths were organized when they were placed in contact with a normal induction field.
They transplanted renal tumor from *Rana pipiens* that had small nuclei into regenerating forelimbs of *Triturus viridescens* that had large nuclei. They utilized a heterotransplantation method and were able to distinguish between the tumor cells of the frog and the blastema cells of the salamander. After the transplanted piece had taken and had begun to grow, the limb was amputated through the tumor. They reported that regeneration was normal in all cases. Histological studies of the regenerate revealed patches of frog muscle, cartilage, and fibrous connective tissue that blended with the corresponding salamander tissue. Therefore, it may be concluded that the inductor of the blastema acted upon the tumor and evoked a normal response in the tumor, since the tumor cells were transformed into normal frog tissue.

The experimental results obtained by Rose and Wallingford (1948) also indicate that neoplasia may be an escape from the control of an inductor. A separation of developmental processes ensues as a result of this failure of control. A mass of undifferentiated cells results. However, if the tumor could be stimulated by a normal inductor, or if the tumor could be brought back into the sphere of influence of an induction field, a control might be exerted over the disorganized mass.

**SUMMARY**

1) The nature of inductor action may be explained by the following theories: a) Axial Gradient Theory, b) Potential Difference Theory, and c) Chemical Theory.

2) Most experimenters agree that the inductor is a diffusible, chemical substance.
3) Experimenters who support the Chemical Theory of Induction disagree as to the chemical nature of the inductor.

4) The inductor, if a chemical substance, may be either sterol-like, carbohydrate-like, or protein-like.

5) There is good presumptive evidence that the inductor is present in every cell of the adult as well as in the embryo. This evidence is obtained from experiments dealing with wound healing, tissue repair, normal cell replacement, regeneration, cancer of the gonads, and adult tissue extracts.

6) The inductor can be liberated from both the adult and the embryo in many ways. Viruses, injuries, carcinogens, cytolysis, and metabolic processes function to liberate the inductor.

7) Liberated inductor may be normal in action, or it may be abnormal in action and produce abnormal growth effects.

8) Neoplasms may be produced by a cellular escape from an induction field.

9) A separation of the developmental processes, proliferation and differentiation, evoked by x-rays, ultra violet light, high and low temperatures, sucrose, and delayed fertilization may lead to the pathological condition of abnormal cellular growth.

10) Teratoma of the adult, as well as of the embryo, may be produced by conditions that disrupt the inductor within the adult and the embryo.

11) A definite functional and perhaps chemical relationship exist between the inductor, certain estrogens, and some carcinogens.
Estrogens, converted chemically into carcinogens, act as disrupted inductors, or disrupt the inductor, and thus they may evoke neoplasms. Such a relationship may account for the high incidence of uterine cancer.

12) The physiological state of a given area constantly changes. Normal inductor may evoke abnormal cellular responses in target areas that are in an abnormal physiological state. Altered inductor need not always be responsible for abnormal cell growths.

**SUGGESTED EXPERIMENTS**

The theory that neoplasms are produced by altered inductors must be proved before it can be accepted. The following experiments may prove helpful in either verifying or nullifying this theory:

1) The transplantation of carcinomas into induction fields (limb buds or optic cups) of the embryo may produce changes in the carcinomas. If the carcinomas are formed as a result of a loss of inductoral control, the placing of the carcinomas under the influence of a control may alter them, and they may proliferate and differentiate in a normal manner.

2) Assuming that a neoplasm is the result of a cellular escape from the influence of an induction field, it may be possible to alter a carcinoma by transplanting an induction field onto the carcinoma. If the inductor is a diffusible, chemical substance, it should exert its influence on the carcinoma and the carcinoma should proliferate and differentiate. However, the target area which, in this case, is the carcinoma, may be
in such an altered condition that it may not be receptive to the transplanted induction field.

3) If neoplastic cells contain disrupted inductor, they should be able to exert an influence on normal cells and cause the normal cells in the immediate vicinity of the neoplastic cells to become abnormal. Pluripotential cells (fibroblasts, mesenchymal, or blastema cells) could be transplanted into a carcinoma, and these transplanted cells might be altered by the disrupted inductor and develop into neoplastic growths.

4) Isolation of the natural inductor from an induction field, and the subsequent application of this inductor to neoplasms, and the possible alteration of these neoplasms to normal cells by this inductor would greatly aid the theory that neoplasms are produced by an altered inductor. The difficulties in isolating the natural inductor are many. The greatest difficulty would be in proving that the inductor substance is the natural inductor, since many substances can induce neural tube formation in the embryo, and these substances need not be of animal origin.
ABSTRACT

The inductor is a substance that is found in the embryo. It functions to evoke cellular proliferation and cellular differentiation. The inductor is more active in some areas of the embryo than in other areas. These active induction areas may be called induction fields.

The inductor was discovered in the embryo. Spemann, Vogt, and Mangold made significant contributions to the field of experimental embryology. The techniques developed by Vogt were supplemented by those developed by Spemann, and an inductoral process was demonstrated by Spemann.

Three theories have been proposed to explain the nature of inductoral action. The Axial Gradient Theory was put forth by some investigators to explain the inductoral process. Other investigators maintained that the inductoral process depended upon an electrical potential. These investigators supported the Potential Difference Theory of induction. These two theories lack the support of most experimenters. Many phenomena cannot be explained by either the Axial Gradient Theory or by the Potential Difference Theory. A Chemical Theory of inductoral action was proposed. Most experimental evidence supports the theory that the inductoral process depends upon a chemical action.

There is strong presumptive evidence that the inductor is not destroyed when the embryonic processes cease. The inductor may be present in every cell of the adult. The evidence from normal cell replacement and from wound healing supports the theory that the inductor is present in the adult. The experimental results from regeneration
experiments, as well as the experimental results with adult tissue extracts, likewise support the theory that the inductor is present in the adult. There is also strong presumptive evidence that the inductor is present in the adult from the results obtained by experiments involving cancer of the gonads.

The exact nature of the inductor is not known. The inductor may be either a sterol-like, carbohydrate-like, or a protein-like substance. There is good experimental evidence to support each of these three theories.

The inductor may be liberated in many ways. It is thought to be released by viruses, carcinogens, cytolysis, and injury. It may also be liberated by metabolic processes. Many inductoral effects can be produced by synthetic inductors. The synthetic substances used to evoke an induction may either act as the natural inductor or cause the liberation of the natural inductor by injury or cytolysis.

A neoplastic growth is one that exhibits proliferation, but it does not differentiate. Neoplastic growths may be produced by a cellular escape from the control of an induction field. When an induction field fails to control cellular proliferation and differentiation, but controls either one of these processes, there is a separation of the developmental processes. A separation of the developmental processes may result in a tumor or a cancer. Tumors are produced experimentally in embryos by processes that disrupt the normal action of the inductor. X-radiation, high and low temperatures, and delayed fertilization are a few of the processes that are used to separate the develop-
mental processes and produce neoplasms. Teratoma of the adult and of the embryo can be explained by the separation of developmental processes within the adult and the embryo. The inductor may have been unable to exert its regulating and coordinating action.

There are many similarities between neoplastic tissue and embryonic tissue. Many substances can induce neoplastic growth. Many substances can induce normal embryonic growth. Some substances possess the unique capacity of inducing embryonic growth as well as neoplastic growth. Estrogens and certain carcinogens can act as estrogens or carcinogens, but they may also act as inducers. Some carcinogens can evoke estrogenic effects. Estrogens can act as carcinogens. The effects, which these substances produce, depend upon the dosages of these substances used. It is also possible to change certain estrogens into carcinogens. Neoplastic growths may be produced by estrogens that have been altered chemically, and these altered estrogens may induce abnormal growths.

It is important to consider the target area that is stimulated by the inductor. Normal inductor may act upon a disrupted target area and evoke an abnormal response. The nature of the inductor, as well as the nature of the target area, must be considered when the action of a carcinogen upon a tissue is studied.

Regenerating tissue is similar to embryonic tissue. The regenerated tissue is stimulated by the inductor that is probably present in the adult. The inductor in an adult regenerate can alter tissue that has escaped from the control of the induction field. Such tissue can
be induced to form normal tissue by the inductor present in the regenerate.

Neoplasms may be produced by an escape of one or more cells from the control of the residual embryonic inductor present in the adult. Such escaped cells proliferate wildly, but they do not differentiate and a neoplasm results. There is also the possibility that neoplasms may be produced when the inductor is disrupted so that it cannot regulate mitotic division and differentiation. There is the further possibility that the physiological state of certain cells may be altered. These altered cells may not respond to the stimulation of the normal inductor in the usual manner, since they may proliferate and not differentiate. A neoplastic growth could then result.
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