THE BORDERLAND OF EMBRYOLOGY AND PATHOLOGY

*The Middleton-Goldsmith Lecture**

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INTRODUCTION

WHILE on the one hand pathology is, rightly enough, the handmaid of medicine, on the other hand she is also a science in her own right. We pathologists are thus not only technicians and advisers to the clinician; we are, or should be, also biological scientists, interested in the fundamental properties of living matter in both disease and health. It is good for us periodically to leave the hospital ward and laboratory, and to take a birds-eye survey of progress in related branches of biology which, we may be sure, will have important bearings on our own. One of the most relevant of these is *embryology;* and my intention in this lecture is to show the significance for pathology of discoveries made by experimental embryologists since the beginning of the present century.[1]

Epigenesis versus Preformation: Of fundamental significance for both embryology and pathology is the concept of *epigenesis*, namely that the egg is a simple structure which does not contain any kind of preformed representation of adult structure and that embryonic development consists in a progressive creation of complex structure stage by stage. This principle was clearly enunciated by Aristotle in the fifth century B.C., and again 2,000 years later by William Harvey who in his "De generatione animalium" (1651) said of the egg "no part of the future offspring exists *de facto*, but all parts inhere *in potentia*." Again, vertebrates "are made by epigenesis, or the superaddition of parts." . . . "A certain order is observed according to the dignity and use of parts, . . . as a ship is made from a keel, and as a potter makes a vessel."

As we shall see, modern experimental embryology has fully con-

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firmed the principle of epigenetic development. Healthy eggs are *not* predetermined to grow into healthy adults, for various injurious agencies in the environment of the egg and embryo can interfere with its development and lead to malformations or other anomalies. Most of the recent experimental work in this field has been done on amphibian embryos, so that it is necessary to recall briefly the early development of the amphibian egg.

Early Embryonic Development

The Amphibian Egg: Even before fertilization the frog's egg has polarity; one hemisphere is pigmented, the other pale. The pigmented pole will become the future head end of the embryo, and the pale pole laden with yolk will become the hind end. Apart from this polarity, the cytoplasm of the egg is undifferentiated.

Fertilization does three important things; it brings into the fertilized egg the paternal hereditary factors; it activates the egg to begin its development, and it determines the plane of bilateral symmetry. The mid-ventral line of the embryo forms at the meridian where the sperm enters the egg, while on the opposite or dorsal side, a little below the equator, there appears after fertilization a grey crescentic area which is of great importance in subsequent development. After fertilization then, the zygote, though still an undivided cell, has all its main axes and its right and left sides determined.

Segmentation now ensues; successive mitotic divisions convert the fertilized egg into a berry-like cluster of smaller cells or blastomeres, a *blastula.* The cells of the pigmented hemisphere of the blastula are smaller than the yolk-laden blastomeres of the lower hemisphere; yolk appears to retard cell-division.

A highly important step in differentiation now takes place in the region of the grey crescent. The surface cells of the cranial hemisphere proliferate rapidly, spread downwards, and invaginate themselves into the interior in the region of the grey crescent (Fig. 1). This invagination, called *gastrulation,* produces a cavity, the archenteron or primitive gut, which opens to the exterior by an aperture, the blastopore. The cranial or dorsal lip of the blastopore is the region of most active invagination, and is a region of fundamental importance in early development. The diving in or invagination of surface cells at the dorsal lip is an autonomous process, for isolated pieces of the lip, when transplanted

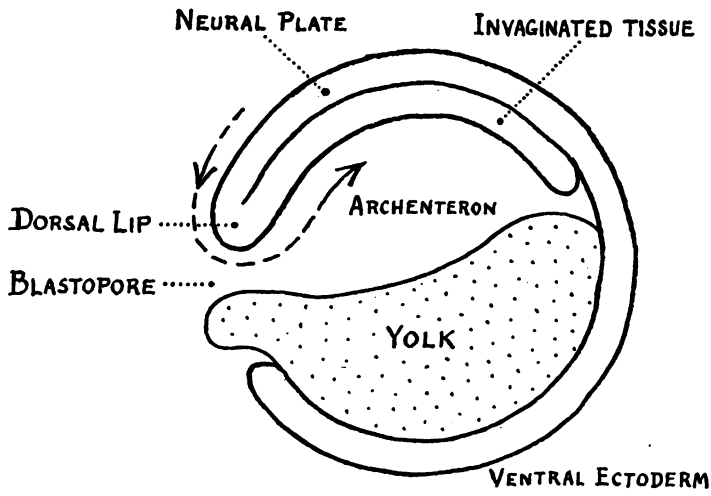

Fig. 1—Diagrammatic mid-line section of frog gastrula, showing invagination of tissue at dorsal lip of blastopore.

to the surface of another embryo, promptly dive inwards in the same way. The cells which invaginate in the mid-line at the dorsal lip give rise to important axial structures of the embryo, the primitive gut roof, the notochord and the paraxial mesoderm or future myotomes. Meanwhile, internally the entoderm and mesoderm are becoming differentiated; so that gastrulation finally results in the delamination of the primary germ layers, the ectoderm externally, endoderm lining the archenteron, and the mesoderm between.

Presumptive Organ Regions in the Blastula: It is possible to map out on the surface of the blastula the regions which normally will become the various organs of the future embryo. By making small injuries or by staining small patches on the surface of the blastula with intra-vitam stains, and then following the subsequent movements and fates of the injured or stained regions, the amphibian blastula has been mapped completely in terms of organ rudiments. We may call these areas *presumptive organs*—presumptive notochord, brain, spinal cord, and so on. The distribution of these presumptive organ areas on the surface of the blastula is quite unlike their adult relationships, which are achieved by the extensive mass movements during gastrulation.

After gastrulation and delamination of germ-layers are complete, the arrangement of organ rudiments is essentially that of the adult. The

plate of neural tissue which now lies along the dorsal side of the gastrula soon presents two parallel longitudinal folds which rise up and fuse dorsally to enclose the neural tube. When this has lost its connection with the surface, the entire surface of the embryo is clothed by epidermis, and the rudiments of all other structures lie internally in roughly their permanent positions. From the time the neural folds appear, the young embryo, which meanwhile is undergoing elongation, is called a *neurula.*

Early Plasticity and Later Non-plasticity or Chemo-differentiation of Tissues: When we speak of the "presumptive" organ rudiments in the blastula, we imply that, given normal development, the regions in question are destined to become particular organs. By transplantation experiments, however, it is found that, up to the middle stage of gastrulation, the prospective fates of most regions are *not* irrevocably fixed. A piece of presumptive neural tube, taken from its embryo and grafted into the side of another, will differentiate into epidermis in accord with its new surroundings, or conversely, a piece of presumptive epidermis grafted into the presumptive neural region of another embryo will differentiate into part of the brain, spinal cord or eye according to its position. Even the germ layers are interchangeable in the blastula or early gastrula. Up to the middle stage of gastrulation, then, the tissues are still plastic or undetermined as regards their final differentiation; each part has potentialities for differentiation much wider than it ever displays in normal development.

During later gastrulation, however, the various regions begin to lose their plasticity. Their prospective differentiation becomes irrevocably determined, and a piece of any given presumptive rudiment, wherever grafted, will now differentiate only into the tissues of that rudiment. A presumptive limb area from a late gastrula or neurula, grafted into an abnormal situation, will continue to form a limb; presumptive epidermis will proceed to form skin wherever it may be transplanted; and a presumptive eye rudiment grafted into the body cavity will produce an eye there. Some change, as yet invisible and presumably chemical in nature, has taken place in each region and has fixed its prospective fate. This change, which precedes visible histological differentiation, is called *chemo-differentiation.* It results in the embryo becoming a patchwork or mosaic of separately determined, though to some extent overlapping, regions; and it is a progressive process, the mosaic coming

to consist of more and smaller pieces as development proceeds.

The Primary Organizer and Axiation: One region of prime importance in the fertilized egg and blastula is exempt from the rule that the different parts of the early embryo are indeterminate or plastic. This is the grey crescent, which gives origin to the dorsal lip of the blastopore, and which from the beginning is destined irrevocably to invaginate beneath the surface and to form the essential axial structures.

The grey crescent or dorsal lip is remarkable, not only because of its inflexible presumptive fate, but also because it has the power of inducing neighboring plastic tissues to disregard their former presumptive fates and to participate in the formation of the main axial and paraxial structures of an organized embryo. Thus, as the classical experiments of Spemann and Mangold (1924)[2] showed, if a small fragment of dorsal lip is grafted into the front or side of another embryo in the blastula or early gastrula stage, it at once invaginates itself and induces the neighboring host tissues to take part in forming a secondary embryo in the body wall of the host embryo. The grafted dorsal lip tissue itself always forms the notochord and usually some of the paraxial mesoderm of the secondary embryo; but the plastic host tissues around, under the inductive influence of the grafted dorsal lip, may produce neural tube, eyes, ears, mesodermal somites, and pronephric tubules. Because of its remarkable power of inducing the formation of the main axial and paraxial parts of an embryo, the dorsal lip tissue was given the name of "*primary organizer.*"

As might be suspected, the organizer is of fundamental importance in normal development, to which indeed it is essential. An egg or blastula deprived of its grey crescent region, or a group of blastomeres isolated from a blastula before they have been affected by the primary organizer, grows and differentiates into a variety of tissues but these form only an unorganized medley. The differentiations attained by such unorganized masses of cells must depend on "labile determinations" of the cells themselves. The organizer is then a directive centre, dictating the fate of neighboring tissues, and integrating all the main changes in early development, particularly those involved in gastrulation and axiation.

One of the most immediate effects of the primary organizer is to induce the formation of the neural plate and tube in the overlying ectoderm. All of the gastrular ectoderm underlain by invaginated

organizer—notochord and paraxial mesoderm—will become neural. Further, the organizer's inductive effects on the overlying ectoderm show distinct regional differences. The first tissue to be invaginated at the dorsal lip of the blastopore reaches furthest forward into the head region, and this tissue induces the formation of cephalic structures—brain, eyes, ears—from the overlying ectoderm. Later invaginated "trunk organizer" will induce only spinal cord and not brain or eyes.

The Nature of the Primary Organizer: The action of the amphibian organizer is *not* species-specific; it can induce the formation of a secondary embryo even when grafted into an embryo of a species different from its own. Organizer material killed by heat, drying or alcohol still has inductive power; so also has a piece of agar or gelatine which has lain in contact with inductive tissue. Cell-free extracts of organizer material are potent, and the solubilities of the active ingredients suggest that they are sterols. Clearly then the activity of the organizer is due to chemical substances which it elaborates. Future research must isolate and identify these substances, and investigate the conditions under which they are active or inactive.

The Organizer in Birds and Mammals: In birds and mammals the homologue of the amphibian blastopore is the *primitive streak* along with the *primitive node and pit.* These structures give rise to the notochord and paraxial mesoderm; and experiments prove that the avian primitive streak has inductive powers similar to those of the amphibian organizer. Like the latter also, it is not species-specific, and it retains its activity after coagulation by heat.

Embryologists have long known that Hensen's node or primitive knot at the anterior end of the avian or mammalian primitive streak is an important centre of growth. From it the notochordal or head process invaginates and grows forward beneath the ectoderm. The primitive pit, which appears just behind the node and which becomes the neurenteric canal, is the homologue of the blastopore, and Hensen's node is its dorsal lip. The lateral lips and their prolongation posteriorly, the primitive streak, are the main growth centres of the embryonic mesoderm.

Secondary Induction; Dependent Differentiation: The dorsal lip of the amphibian blastopore or the avian primitive streak is the *primary organizer,* because it initiates the whole complex process of somatic organization and because its inductive capacity is a primary one not de-

pendent on any earlier organizer. But, after primary organization has been determined during gastrulation, many tissues other than the derivatives of the primary organizer play the part of *secondary organizers* in that they exert inductive influences, presumably chemical in nature, on neighboring plastic tissues, which therefore undergo *dependent differentiation.* The classical example (Spemann, 1901[3]) is the dependence of lens formation on the presence of the optic cup. The lens of the vertebrate eye develops as a localized thickening of the embryonic ectoderm overlying the optic cup, the outgrowth from the brain which gives origin to the retina and optic nerve. If the optic cup is removed before the lens has formed, no lens appears; and an optic cup transplanted under the skin elsewhere induces the formation of a lens from the overlying epidermis.

There is no doubt that similar inductive influences operate in many developing organs. The post-gastrular embryo is a complex mosaic of interacting parts. The fates of most of these are already determined in broad outline, but there are still subordinate tissues the complete differentiation of which is dependent on the proper development of other tissues. These latter are *secondary organizers,*[4a] and their inductive effects on neighboring parts depend, like the inductive effect of the primary organizer, on chemical substances elaborated by them. "There is, then, a whole hierarchy of organizers or morphogenetic hormones functioning in development." (Needham[4b]).

Experimental Extirpation of Parts of Embryos: If an amphibian embryo after gastrulation is transected each part will continue its prospective development, the front part forming a head only and the hind part a trunk and tail only. If in the early tail-bud stage the bud is cut off, a healthy organism will develop but with a permanently deficient tail. Removal of rudiments of eyes, limbs, spinal cord, pituitary or other parts results in permanent lack of these structures later. By appropriate operations on the early embryo almost any desired defect can be produced in the developed organism.

Experimental Transplantation of Organ-Rudiments: Almost all organ-rudiments and tissues of amphibian, avian or mammalian embryos are capable of self-differentiation when suitably transplanted or when suitably cultivated *in vitro.* In the chick, grafting of embryonic rudiments on the chorio-allantoic membrane of another egg has shown that isolated ears, eyes, part or whole limb-rudiments, parts of brain and

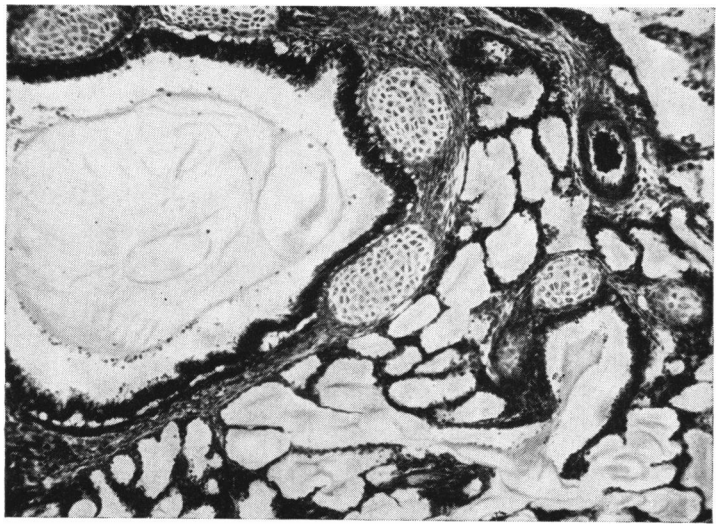

Fig. 2—Well differentiated lung tissue with bronchi and alveoli, 2 weeks after intracerebral transplantation of lung rudiment of a 6 mm. rat embryo.

spinal cord, lung, etc., grow and differentiate well. In amphibia the mosaic determination of various regions has been demonstrated mainly by grafting them in abnormal positions in other embryos, in which as they develop they attain their proper prospective fates independent of their new environment.

Some experiments of my own[5] proved very successful in demonstrating the powers of self-differentiation of isolated tissues and organ-rudiments from mammalian embryos. Finely minced tissues or selected whole organ-rudiments from rat embryos were implanted through a wide-bore needle into the brain or cranial cavity of young adult rats. The host rats were killed after intervals of from 1 to 3 months and the grafts studied. It was found that almost all kinds of embryo tissues grew and differentiated as well in their foreign sites as they would have done in normal development. Good growth and differentiation of the following structures were obtained: skin, sebaceous and sweat glands, hairs, tongue-epithelium, conjunctiva, whole teeth, cartilage, pieces of bone, bone marrow, whole bones, bronchi and lung, stomach, pancreas, intestine, prostate, lachrymal gland, mucous glands, salivary glands, thyroid, pituitary gland, testis and epididymis, thymus, striated muscle,

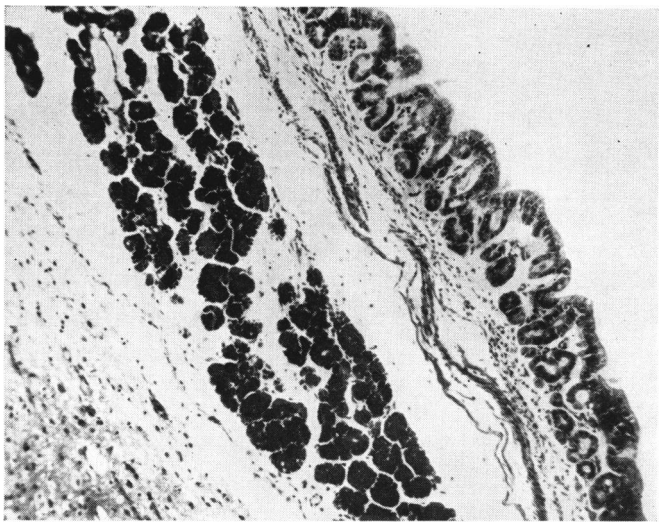

Fig. 3—Well differentiated stomach and pancreas 2 weeks after intracerebral transplantation of rudiments of these organs of a 6 mm. rat embryo.

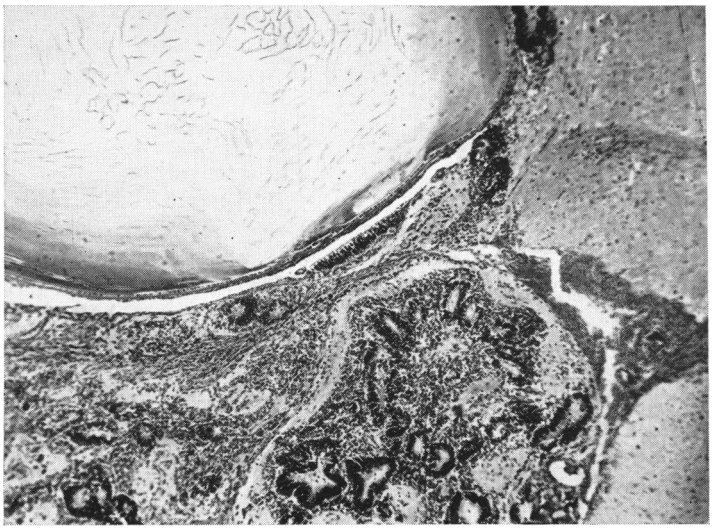

Fig. 4—Lens and retinal tissue 4 weeks after intracerebral transplantation of eye rudiment of a 10 mm. rat embryo.

lens, retina and cochlea (Figs. 2-4). In all experiments the differentiated tissues which developed in the grafts corresponded strictly to the region of the embryo which had been transplanted.

It may be mentioned here that the experimental implantation of embryo tissues into adults has long been practiced by experimental pathologists in the hope of verifying Cohnheim's hypothesis that tumors arose from embryonic "cell-rests," or in the hope of shedding light on the genesis of teratomas. Both of these hopes are false: grafted embryo cells do not give rise to tumors; and the mixtures of tissues in differentiating embryo grafts are not comparable with spontaneous teratomas, the genesis of which must be sought rather in disordered chemistry in the early embryo.

Cultivation of Organ-Rudiments in vitro: Many kinds of organ-rudiments have been grown successfully in culture media. Amphibian notochord, neural tube, muscle, skin, gut, liver, pancreas, etc., have shown normal differentiation during their periods of survival in culture. Pieces of gut differentiated in culture have shown peristaltic movements, and pieces of heart have begun to pulsate. Whole heart-rudiments have developed sinus, auricle, ventricle and aortic bulb. Organ-rudiments from chick embryos and from mammalian embryos also have shown successful growth and differentiation *in vitro*. For example, the early cartilaginous rudiments of the chick's femur will grow into a recognizable femur increasing its bulk 30-fold or more and showing normal ossification.

Teratology, the Pathology of the Embryo

Teratology Distinguished from Foetal Pathology: In 1904 Ballantyne[6] stressed the distinction between the pathology of the foetus and the pathology of the embryo. Broadly speaking the foetus, with its differentiated parts and tissues, resembles an adult in miniature, and the reactions of its tissues to injuries, poisons and infections are similar to those of the adult. But the embryo is structurally and functionally unlike an adult. "The great, almost the only, function of the embryo is to form tissues and organs, or, in one word, organogenesis." Hence, the pathology of the embryo is unlike that of the foetus; it is *teratology*. Just as the chief activity of the embryo is the formation of parts and organs, so the effects of pathogenic agents in the embryo are malformations of parts and organs.

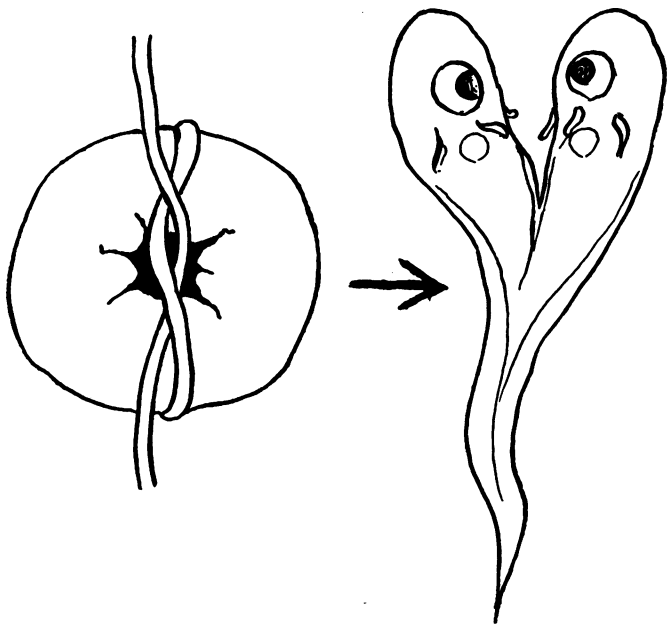

Fig. 5—Diagram of Spemann's experimental production of anterior duplication of newt embryo by ligature of blastula vertically through the blastopore.

This conception of the distinction between embryonic and foetal pathology has been confirmed by experimental embryologists. Many kinds of malformations can be produced experimentally by exposing embryos of suitable ages to abnormal environments. The abnormal conditions capable of causing malformations are very diverse, and include many agents which produce "diseases" in the developed organism, e.g., trauma, oxygen deficiency, abnormal temperatures, various chemical substances, and pathogenic organisms. Let us look at some of the experimental results, commencing with the grossest malformations of all, double monsters.

The Experimental Production of Double Monsters and other Major Malformations: Double monsters have been produced in many different ways, the chief of which are as follows:

(a) *By interference with the zygote during early segmentation,* e.g., in Amphioxus by shaking and disarranging the blastomeres, and in the frog by inverting the egg at the 2-cell stage.

(b) *By partial constriction of the embryo during gastrulation.* Spemann showed that if the early newt embryo is constricted in the median plane during gastrulation, the resulting embryo will show anterior duplication, i.e., it will have two well-formed heads joining with a single hind end at varying levels. The explanation is clear: the invaginating organizer, moving anteriorly from the dorsal lip of the blastopore, meets an obstacle in the constriction and therefore bifurcates, one-half of it going forward on either side of the constriction. The Y-shaped invaginated organizer induces a Y-shaped embryo, with duplicated head regions (Fig. 5). If the plane of constriction of the blastula is not accurately median, but slightly oblique, then one head of the resulting double monster is normal and the other is incomplete and usually cyclopic. The greater the obliquity of the constriction, the greater the imperfection of the malformed head.

(c) *By oxygen deficiency or lowered temperature.* By these means, Stockard[7] caused retardation of development during early cleavage of trout and minnow embryos, and produced a great variety of major malformations, including double monsters, cyclopia, malformed brains, eyes and viscera. These results were traceable to irregular and delayed segmentation of blastomeres occasioned by the developmental arrest.

(d) *By delayed fertilization.* Witschi[8] found that if fertilization of frog's eggs was delayed, various abnormalities resulted. Brief delay resulted in an abnormal sex ratio in the resulting tadpoles, males predominating. Longer delay of fertilization resulted in the production of double monsters and sometimes of teratoma-like growths. The double monsters were traceable to abnormalities of cleavage and to splitting of the blastopore lip at the onset of gastrulation.

All the foregoing methods of producing double monsters involve treatment of the embryos at very early—pregastrulation—stages. "When eggs are treated at later stages, as at the beginning of gastrulation, no double monsters will occur, their moment has passed." (Stockard[7]). Duplication of the axis of the body, of any degree, presupposes corresponding duplication of the inducer of axiation, the primary organizer. Double monsters are malformations of primary axiation and can only be produced therefore by disturbances affecting the embryo prior to establishment of its axis. Once the axis is laid down, the embryo has become a mosaic of organ-fields, the future differentiation of each of which is determined; and no amount of interference can produce more

axis tissue.

Experimental Malformations in the Mosaic Embryo: As already noted, after the stage of mosaic determination of a particular organ-field, extirpation or damage within that field will lead to permanent defects or deformities of the organ. Similar defects can be obtained by oxygen deficiency, lowered temperature or chemical poisons applied to the embryo at appropriate stages during or after gastrulation. By these means, the experimentalist can produce, in amphibian or fish embryos, the main axiation of which has developed normally, cyclopia, deformed mouths, nostrils, gills, brains or viscera. The particular malformations produced depend, not on the kind of abnormal agent used, but on the time at which it is applied.

Thus, experimental work has verified Ballantyne's[6] prescient opinion that malformations "are the results of morbid agents . . . acting upon such parts of the organism as are in the embryonic or formative stage." Stockard[7] expressed the same principle when he spoke of "developmental arrests at *critical* stages, . . . when certain important developmental steps are in rapid progress or are just ready to enter upon rapid changes."

Developmental arrest: Many malformations are developmental arrests in the sense that they consist in non-formation of parts, persistence of parts which should have disappeared, non-union of parts which should have coalesced, or failures of canalization.

A special group of developmental arrests, probably related to deficiency of the circulation in the embryo and early foetus, has been studied by Streeter[9] and by Sir Arthur Keith,[10] namely the deficiencies of the limbs to which the name "intra-uterine amputation" used to be applied in the mistaken belief that they were due to constriction by "amniotic bands." "In these cases sharply circumscribed areas of limb-bud tissue are of such inferior quality that only imperfect histogenesis occurs. Whether injured in some way or defective from the outset, and the latter is probably true, these areas maintain themselves only in the earlier weeks of pregnancy. Already at the fourteenth week they are found as fibrous moribund masses, surviving after the manner of tissue-cultures and sloughing away from the adjoining normal tissues. . . . The defects if present in one extremity tend to be in all four, which would be expected on the basis of a general deficiency in the limb-bud tissue. The defects tend to be in the distal portions of the extremities."

Sir Arthur Keith[10] extended the conception of Streeter's "foetal

dysplasia" to many other kinds of malformations–facial clefts and scars of obscure origin, anencephaly, spina bifida and meningocele; and advanced the view that "all such lesions are caused by a local necrosis probably due to a circulatory failure which may be placental in origin. The failure occurs along marginal areas where capillary formation is in progress. . . . 'Amniotic adhesions' are never formed by a failure in the separation of the amnion from the embryo, but are always produced by and from the foetus–as a result of dysplasia in foetal tissues. They are the result, not the cause, of foetal malformations." Perhaps impaired circulation may underlie also such failures of coalescence as cleft palate, coloboma, hypospadias, and other local arrests of growth movements.

Specificity of Some Factors Causing Malformations: While it is certainly true that identical malformations may result from widely different agents, yet there is also evidence that some of these agents selectively damage certain embryonic tissues.

A good example of this is afforded by the recent discovery that German measles affecting a woman during the first two or three months of pregnancy is apt to cause certain characteristic defects in the child, the chief of which are congenital cataracts, cardiac malformations, deaf-mutism, and microcephaly (Swan[11]). Here then we have an example of an infective disease, the virus or toxins of which have a selective damaging effect on certain parts of the embryo or early foetus; the results are, not the disease rubella as it occurs in the adult or child, but certain localized malformations or dystrophies.

Ballantyne[6] collected much evidence that various kinds of maternal infections or toxic diseases, including syphilis, tuberculosis, lead and other chemical poisonings, could damage the embryo or foetus. He recognized that the commonest result of such maternal diseases was foetal death; but he believed that, should the foetus survive, the effects of the damage inflicted on it during early growth might appear as malformations or dystrophies, admitting that it was difficult to prove the culpability of any particular causative agent. The association of malformation with rubella, just cited, is a clear instance in support of Ballantyne's general thesis, and it should stimulate new and more extensive studies, both clinical and experimental, of the possible influence of all kinds of maternal diseases in producing teratological effects.

Foetal and Post-natal Malformations: While it is true that most major malformations take origin in the embryonic period, we must remember

that the distinction between the embryonic and foetal periods is arbitrary. Many parts and tissues are still immature or "embryonic" in the foetal period, and indeed the differentiation of some parts is not complete until long after birth. Thus, adult structure is not attained in the genital system until after puberty, in the skeleton until the epiphyses unite, and in the jaws until the permanent teeth erupt. The immaturity of such parts in childhood is, as it were, a projection of embryonic and foetal development into post-natal life; and in such parts minor malformations theoretically may, and in fact do, sometimes arise from disturbances of late foetal or post-natal development. For example, destruction of an epiphysis will result in arrest of growth in length of the bone, and the stunted bone is as much a malformation as if it had resulted from damage inflicted on the primordium of the bone in the embryo. So also dentigerous cysts and odontomes are malformations brought about by disturbances of dental development during late foetal or post-natal life. The pseudo-hermaphrodite condition of the genitalia resulting from adrenal cortical tumors in the foetus is another example of a malformation acquired relatively late in intra-uterine life.

Metaplasia

We have seen that, while most of the parts of an early pre-gastrular embryo are completely plastic and are capable of differentiating into almost any tissue according to the environment in which they are placed, after gastrulation they lose this nearly total potency and become determined to differentiate into specific structures of the adult body. But, even in the adult, many of the tissues are not immutable, but are capable of limited degrees of divergent differentiation in abnormal proliferative states—in regeneration and in tumors. These transformations, called *metaplasias*, show that a residue of the plasticity of the early embryo persists throughout life.

We need not spend time on detailed examples of metaplasia, which are familiar to all pathologists. Suffice it to recall that in both inflammatory lesions and tumors glandular or mucosal epithelia of various kinds may become squamous; that bone or cartilage may develop in many soft-tissue lesions, such as scars, sclerotic arteries, foci of chronic inflammation or degeneration, and the stroma of some tumors; that in many rarefactive states of bone, bony tissue often suffers transformation into fibrous tissue; and that bone formed by metaplasia in soft tissues

may contain all of the components of haemopoietic bone marrow, which must also be locally produced. Even smooth and striated muscle fibres are not immutable, for, as Carey's experiments[12] showed, by excessive exercise of the dog's bladder its muscle fibres can become striated.

Many adult tissues, then, possess a potential versatility for aberrant differentiation which they do not display under normal conditions, but which are to be looked upon as adaptations to an abnormal environment. These metaplastic adaptations occur only in proliferating cells; they are, as Nicholson[13] maintained, a form of regeneration with atypical differentiation. The proliferating cells "undergo a true rejuvenescence, and . . . some of the earlier potencies are reacquired." Regeneration is indeed resumed embryonic growth, and metaplasias are the visible evidence of resumed embryonic plasticity.

Embryonic Tumors

Truly embryonic tumors are those which arise in early life from tissues that are still undifferentiated and which continue to proliferate at this embryonic level. They include nephroblastoma (Wilms' tumor or embryonic renal tumor), neuroblastoma, retinoblastoma (retinal neuro-epithelioma), hepatoblastoma (embryonic tumor of liver), and the embryonic sarcomas (often rhabdomyosarcomas) of the urogenital organs of children.

The structure of these tumors can be understood only in the light of that of the corresponding tissues at various stages of embryonic development. Thus, nephroblastomas consist of embryonic and foetal renal tissue, in some cases wholly undifferentiated, in others differentiating into tubules, glomeruli and non-epithelial mesenchymal tissues, these last sometimes showing heterotopic differentiation, e.g., into striated muscle. The characteristic fibrillar rosettes of neuroblastomas are similar to those seen in the developing sympathetic ganglia of the embryo and early foetus. Retinoblastomas reproduce the structures of developing retina; their rosettes consist of small cavities corresponding to those of the embryonic optic vesicle into which rod and cone processes project through a characteristic limiting membrane.

Some of these embryonic tumors are called "mixed" because they show differentiation of tissues which do not occur normally in the organs involved, e.g., striated muscle or cartilage in renal or hepatic tumors. But these heterotopic tissues are only such as can readily be

derived from immature mesenchyme (*vide* Metaplasia above). They are never utterly foreign to the part; e.g., nephroblastomas do *not* contain skin, teeth, central nervous tissue, respiratory or alimentary structures, all of which are common in teratomas. The embryonic tumors thus differ from teratomas in having regionally restricted potencies for differentiation—potencies of no greater range than those of the particular tissue of origin.

The causation of embryonic tumors is, as H. G. Wells[14] pointed out, an interesting subject for speculation. Most of the ordinary tumors of adults arise only after prolonged exposure of the tissues to carcinogenic agents; the embryonic tumors arise during actual organogenesis, and clearly their causes must be sought in disturbances of embryonic chemistry—the chemistry of the organizers or growth-hormones which we discussed earlier. When discovered, however, the chemistry of these disturbances may well shed light on the central problem of neoplasia, namely the very nature of the irreversible change in the tumor cell. For this reason alone, the embryonic tumors and the kindred teratomas are of peculiar interest and merit the closest study.

Teratomas

Like the regionally specific embryonic tumors, teratomas also arise during early stages of development from immature tissues, and many of them (the malignant ones) continue to proliferate at the embryonic level and to produce tissues of all degrees of immaturity. But, unlike the regionally specific embryonic tumors, teratomas contain a variety of tissues quite foreign to the part. In malignant teratomas recognition of these tissues necessitates a good knowledge of the normal histology of embryos at all stages of development. Pathologists unfamiliar with normal embryonic histology have often misidentified various teratomatous tissues, e.g., both embryonic choroid plexus and neuro-epithelial tissue have often been called "adenocarcinoma," and the all-embracing name "embryonal carcinoma" has been used as a convenient label for a variety of undifferentiated epithelial tissues in teratomas.

Many speculative hypotheses as to the nature of teratomas have been advanced; and still the most prevalent view is that they are distorted foetuses included within the host or derived from his or her own germ cells. The falsity of this view is shown by the following facts:

1. By topographical reconstructions of whole teratomas, using the

serial slab method employed by the writer,[15] it is found that teratomas, unlike the most degraded of amorphous foetuses, show no signs of the possession of a vertebrate axis, or of delamination of germ-layers, or of regional distribution of organs.

2. Teratomas, again unlike amorphous foetuses, are *neoplasms* with powers of progressive independent growth, extension and metastasis. The advocates of the "foetus" hypothesis ignore this fundamental attribute.

3. Highly organized parts which may occur in a teratoma, e.g., teeth, a loop of intestine, a piece of cerebellum, or digits (Fig. 6),[16] do *not* presuppose a foetus. A spark-plug is a highly organized structure, but its construction does not presuppose the previous construction of a motor car. Complex parts in teratomas result from the local mutual influences of proliferating plastic tissues on one another—influences comparable to those of the secondary organizers in embryogenesis, whereby one growing tissue chemically induces a neighboring plastic tissue to participate in the formation of an organized composite structure. Careful topographical studies of teratomas enable the histogenesis and mutual relationships of the differentiating tissues to be traced.

In nearly all malignant teratomas containing a variety of embryonic tissues, there are frequently present small or extensive areas of a rather characteristic, though quite undifferentiated, component consisting of an irregular closely aggregated meshwork of plump cuboidal cells, with intervening strands of mesenchyme and blood vessels (Fig. 7). This tissue, which has often been vaguely designated "carcinoma" (especially "embryonal carcinoma") is the most primitive embryonic component recognizable in these growths, and its relationships show that it differentiates into a variety of specific tissues—epidermal, dental, neural, probably respiratory and alimentary, and possibly also mesenchymal. If this view is correct, the undifferentiated tissue in question represents the essential primordial element in teratomas; for, like the tissues of the pre-gastrular embryo, it is totipotent embryonic tissue from which all else that is found in these growths may spring. In malignant teratomas containing it, its proliferation continues at this very early embryonic level, simultaneously with partial maturation of parts of it into specific tissues of various kinds. In benign teratomas, containing only fully differentiated tissues, the whole of the primordial tissue has matured and differentiated with the lapse of time. Benignancy or malignancy in

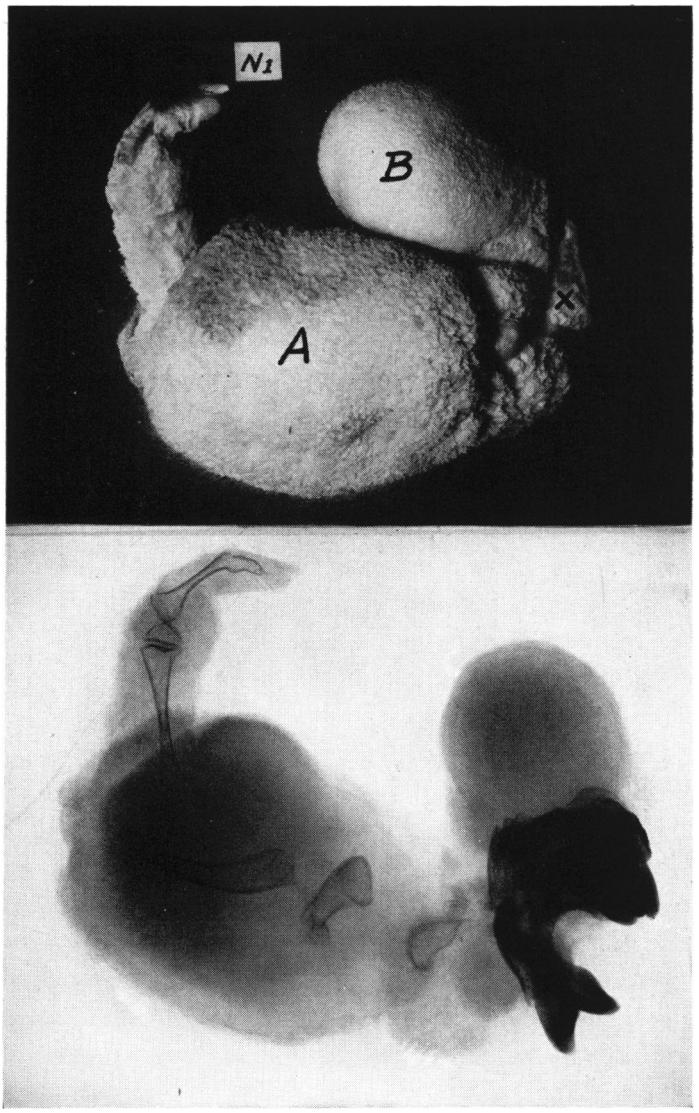

Fig. 6—A digit in a retroperitoneal teratoma. AB is bilobar skin-covered eminence which projected into cyst cavity from pedicle X; N1, a well-formed nail at end of digit. The X-ray photograph shows the phalanges and metacarpal, and also several non-descript bones in the main mass. (Specimen has been described fully by Gale & Willis[16]).

Figure 6 is reproduced with the kind permission of the Editor of the Journal of Pathology and Bacteriology.

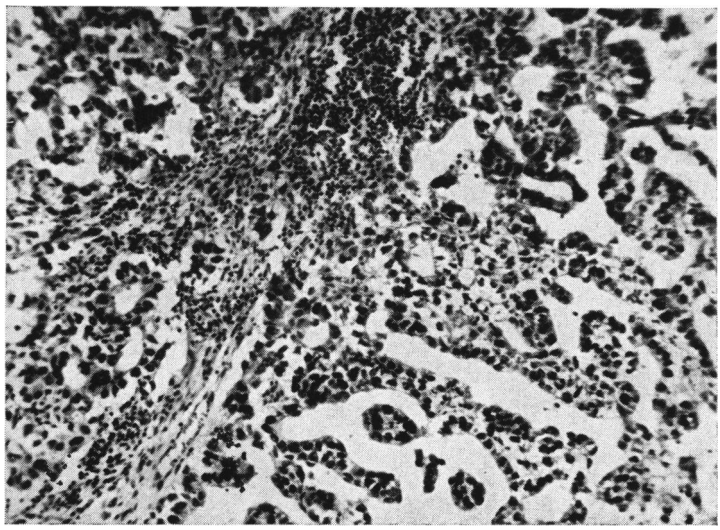

Fig. 7—Undifferentiated embryonic epithelium-like tissue, a frequent component of malignant teratomas.

teratomas is thus related to the success or failure of maturation of their component tissues; and we will understand this only when we learn more of the chemistry of the hierarchy of organizers concerned in embryogenesis. Then, too, we will realize how crude have been our earlier speculations as to the nature and mode of origin of teratomas.

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