EXPERIMENTAL STUDIES ON THE ORIGIN OF VASCULAR ENDOTHELIUM AND OF ERYTHROCYTES

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EIGHTY-NINE FIGURES

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INTRODUCTION

The following contribution represents a résumé of three years of experimental study in which an attempt has been made to analyze certain of the processes involved in the genesis of some of the vascular tissues. For constant encouragement and most helpful criticism I wish to acknowledge my deep indebtedness to Prof. Charles F. W. McClure, under whose guidance the work was undertaken. I am especially grateful for the generosity with which he has furnished me with necessary funds and apparatus for the undertaking of the problem at hand. I have also been so fortunate as to have recourse to the valuable counsel of Prof. E. G. Conklin, whose advice has been of great help to me.

PART I. THE ORIGIN OF ENDOTHELIUM

In many respects the development of the vascular tissue is unique. Its investigation is attended with certain peculiar difficulties which have hindered the solution even of some of the least involved problems in this field of development. Within

recent years the origin of the endothelium which lines the haemal and lymphatic vessels has perhaps been the central problem of anatomical investigation. Over this question there has been an extended controversy; even now it would be premature to say that all the problems confronting us are completely settled.

The principal points of difficulty with which one must reckon in consideration of this peculiarity of endothelial tissue are raised by these questions: Does physical continuity of endothelial cells necessarily imply genetic continuity? Conversely, is it possible that cells out of continuity with already formed endothelium can ever become continuous with and a part of that endothelium? It has been observed that in most classes of vertebrates the site of endothelial formation is the yolk-sac. Later, endothelium is found progressively nearer the embryonic body and finally inside the embryonic body. As has been so many times stated, there are two views concerning the nature of this endothelium which is found associated with the intra-embryonic tissues. One view is that the earliest vascular tissue grows toward the embryo, and on reaching the embryo's body permeates the intraembryonic tissues in a centrifugal manner, forming the entire lining of the haemal and lymphatic systems. This view has been designated as the angioblast theory of Wilhelm His. Let us consider a few quotations from certain of the supporters of this theory; in this manner we can study first-hand the conceptions of these observers concerning the implications of this so-called ingrowth or extension theory.

I. THE ANGIOBLAST THEORY

Minot's conception of the angioblast (Keibel and Mall, *Human Embryology*, vol. II, pp. 498-9) is as follows:

Comparative embryology teaches us that the first vessels appear on the yolk-sac collectively and at one time. They form a unit anlage, which we call briefly the angioblast according to the suggestion of His. . . . I am inclined to think that the angioblast . . . forms itself not through the transformation of mesodermic cells already present, but from the layer of yolk-cells, and from a reticulate grouping of themselves between the middle and lower germ-layers. The angioblast probably maintains its complete independence throughout

life. In other words, it is probable that the endothelium of the blood-vessels (and of the lymph-vessels) and the blood cells at every age are direct descendants of the primitive angioblast.

The following is from Evans (Keibel and Mall, vol. II, p. 571):

In embryos of the higher vertebrates the first cells which can be identified as standing in any relation to the vascular system appear in the form of localized thickenings of the extra-embryonic mesoderm lying next the entoderm of the yolk-sac. These constitute the so-called vascular anlagen and typically undergo a gradual differentiation into two or more definite cell-types, blood cells on the one hand, and endothelial cells on the other. . . . It is important, then, to distinguish vessels which have arisen through sprouting of other vessels in contrast to vessels whose endothelium has been contributed directly from the neighboring mesoderm. Even on the yolk-sac these latter vessels are not numerous, for they occur only at the site of the so-called anlagen, and the main mass of the vitelline capillary plexus arises from the extension and frequent anastomosis of these primary vessels. . . . In birds it has been possible to establish beyond all doubt that most of the aorta descendens is formed from the medial margin of the vitelline capillary plexus.

Bremer (3) agrees with the angioblastic view. He believes, however, that the injection method is inadequate for mapping out the extent of the vascular tissue, even in case of the caudal aorta, as the establishment of its lumen is not a continuous process, so that isolated cavities actually exist, but the cells of their walls are in genetic continuity. He states (p. 114) that "it was the observance of these isolated spaces which later fuse to form large vessels, that lead to the often repeated statements of Rückert and Mollier and others that the dorsal aortae arise in situ from cells of the mesoderm."

In 1914 (4) his conception of the angioblast is much modified (p. 462):

I think it well at this point to define more accurately what I mean by angioblast cords, especially since I believe that their recognition may help to explain the frequently described endothelial spaces unconnected with any injectible vessel. The angioblast cords are apparently solid cords of cells connected end to end or in small groups, running between the processes of the surrounding mesenchyme cells, when these are present, often touching them, without actually fusing with them. The diameter of the cords is never as small as that of the

mesenchymal process, though it is often less than that of the cord nuclei. The cords tend to form nets by anastomosis of larger mesh than the mesenchymal net, and angiocyts wherever space is given. They are usually sharply defined from the surrounding tissue, and may show an extraintimal space.

In this sense the angioblast is not necessarily the unit vascular anlage which grows into the embryo from the yolk-sac. Its only prerequisite is that it shall grow continuously (from one or many sources) through the embryonic tissue as 'solid cords of cells.'

It is to be hoped that the term 'angioblast' may never be applied to any structure except to this theoretically early differentiated, precociously segregated, yolk-sac, unit vascular anlage which has been claimed to give rise to all the vascular tissues. If no such exclusive and sole 'unit anlage' exists, then the term needs and deserves to be discarded. It has been used in the literature for such a great number of years that its meaning is very definite and well established. The term is so intimately related to the concepts of specificity and ingrowth from the yolk-sac of all vascular tissues that it can never, with propriety, be attached to any embryonic structure except the early yolk-sac vessels. It is to be hoped that no observer will ever find it necessary to designate individual mesenchyme cells as 'angioblasts,' even provided he should become convinced that such cells are vasofactive. The term 'angioblast' implies a 'unit anlage;' it implies a collectively multicellular tissue, and therefore can not properly be used in connection with a single cell. If there be such a thing as angioblast, there is but one angioblast and it has but one method, place, and time of origin. To no isolated cell should be attached a term which for decades has been applied to a group of yolk-sac blood-vessels. Strictly speaking it would be inconsistent to designate as 'angioblastic cords' any solid forerunners of intraembryonic vascular tubes, even though the ingrowth theory were correct. Angioblast can not properly be applied to solid or tubular endothelium which has arisen by sprouting; the term implies the original anlage which does the sprouting, and which does not, according to the angioblast theory, arise from preexisting vascular tissue; it does not include the sprouts, or the sprouts of sprouts.

Finally the view of Rabl might be mentioned. He is responsible for the dictum "Endothel stammt nur von Endothel." Taken literally such a statement would relegate vascular endothelium to a category in which the germ cells, and perhaps some of the most primitive organisms are generally regarded as unique because of their supposed immortality.

II. THE LOCAL ORIGIN THEORY

The view opposed to the angioblast theory is that of local origin. According to this view, mesenchyme may, in practically any region of the body, transform into vascular tissue. The cells which bound an intraembryonic blood-vessel are not in direct lineage with those which line the early vessels on the yolk; they have not come into being as ingrowths from the early yolk-sac vessels or 'angioblast;' they have not necessarily come from preëxisting endothelial cells, though some of them may have had such an origin, inasmuch as local origin does not preclude the possibility of growth during or following the process of local vascular formation. Additions to endothelium already formed may take place by (1) proliferation of cells already formed, (2) addition of single mesenchyme cells, (3) addition of solid cell aggregates, (4) addition of already formed endothelial cavities the lining cells of which have differentiated locally, in and from the mesenchyme, and (5) by the active migration and alignment of single mesenchyme cells to form vascular cavities. The local origin theory holds that blood-cells are not necessarily descended from a primitive yolk-sac 'angioblast,' but that mesenchyme within the embryonic body is capable of giving rise to blood cells. Advocates of the local origin theory do not believe that the vascular anlagen are necessarily differentiated at a very early stage of development, as claimed by His, or collectively and at one time as stated by Minot; advocates of the mesenchymal theory recognize that there are certain regions in which a precocious production of vascular tissues takes place, but they claim that such regions are not the only regions in which such tissues are formed. Advocates of the local origin theory recognize various intraembryonic regions in which there is a first-hand production of vascular tissues, even relatively late in ontogeny, and quite independent of such processes in the yolk-sac. The angioblast theory regards endothelium as a tissue of high specialization, quite foreign in nature to mesenchyme and quite removed from it genetically. The local origin theory

claims that mesenchyme can transform into endothelium, and that endothelium can transform into mesenchyme.

The theory of local mesenchymatous origin of endothelium dates back to the work of Goette (15) and Reichert (56). Within more recent times, Rückert and Mollier (60) and their students have chiefly constituted the European School who maintain that endothelium develops *in situ*. Maximow (31), Bonnet (2), von Felix (13) and others have also supported this view. In this country, Huntington (19, 20) and McClure (32-37) and their students have stood practically alone as sponsors of the local origin theory.

It is of interest to note some of the methods which have been employed by the advocates of these conflicting views. Investigations supporting the angioblast theory have sometimes been based on studies of serial sections, frequently on relatively late growth stages in the living tissues of amphibian larvae and other tissues, and especially within recent times, on injections. Certain objections to the results obtained by these methods have been raised by the opponents of the angioblast school. Especially Huntington, McClure, and Schulte have urged that intravital study of late capillary plexuses, as has so often been made, [Platner (47), Remak (57), Kölliker (25), Langer (26), Clark (7), etc.] is merely a study of endothelial proliferation and not a study of its genesis. To quote McClure (36), pp. 61-2):

We all recognize the fact that endothelium, like other tissues of the body, is capable of growth after it has once been formed. In no other manner could we account for the increase in size which blood-vessels undergo in the embryo. . . . It is also possible for anastomoses to be formed between different vessels by means of growth or sprouting of their endothelial walls so that, in some cases, an increase in their extent through growth may actually take place. . . . From whatever standpoint it may be considered, the growth of endothelium is of secondary significance as regards the problem at hand, since the main question at issue does not concern the possibility that endothelium may or may not grow, but rather how the endothelium is formed that does the growing.

The injection method has also been criticized as a method capable of giving only one result—a negative result with refer-

ence to the possibility that non-continuous vascular lumina exist. Huntington and McClure have repeatedly called attention to the fact that injection will do nothing more than demonstrate continuous cavities. Those who believe the injection method to be adequate, maintain that any cavity in the uninjectible periphery is necessarily a non-vascular cavity or an artifact. In case of the early vessels in the chick, the injection method should be able to demonstrate all endothelium from the moment that the independently formed portions of the angio-blast or 'unit anlage' have become confluent. In this connection Schulte (63, p. 25) states:

From this moment . . . there is a sudden and abrupt end to the production of discrete anlages, contrary to what one would expect from the general transitions in natural processes.

But in view of the many anlages admittedly present in the splanchnopleure and in the embryo, and of transitions almost universally observed in natural processes, the possibility must be conceded that a belated vesicle or two of endothelium might conceivably escape a sudden annexation to the injectible system. It becomes of interest to determine its status after injection has been practised and it has failed hypothetically to be injected. A moment before, had it been observed in vitro, or had it been sectioned, it would have appeared like any other of the discrete anlages in the 'anlage.' But the test of injection made, it at once ceases to have a future, it has become an artifact, or if pertinaciously insisted upon, a Mayer-Lewis anlage. Because . . . it failed of concrescence prior to the moment of injection it is absolutely devoid of vascular potentialities and could never, had the injection been omitted, have joined its fellows and participated in the formation of the vascular system. . . . (p. 7). We may recognize, or refuse to recognize the uninjectible periphery. If we recognize it, the method of injection becomes not only partial and incomplete, but subordinate to the methods which reveal all its findings and in addition, enlarge our field of observation.

. . . (p. 27). To . . . insist that the limited field explored by injection is alone accessible to investigation is not to assert the primacy of the injection method, but to fall back upon an ancient logical device known as *petitio principii*. . . . (p. 26). There is, however, no reason to assume that injection has reached the acme of delicacy, great as is the skill which has been developed in its use. It is conceivable that some day it will be possible to inject a portion of the 'anlage' before its admittedly discrete vessels have fused—even a small fragment might be injected alone, the dorsal aorta in the head, say, or the umbilical vein. This would then become to the injectionist the only source of the vascular system. The un.injected parts of the

anlage would be demonstratedly not vascular anlagen at all, but artifacts or 'Mayer-Lewis anlagen,' and no study by slide or section could establish their right to serious consideration.

It is of interest in this connection to note that McClure (33, 34, 36) has been able actually to inject a discrete lymphatic anlage—the sub-ocular lymph sac in the trout—independently of the main systemic lymphatics, veins, and arteries, yet it is inconceivable that these latter vessels, as revealed in section, could be regarded as non-vascular spaces.

In defense of the injection method, E. R. Clark (8) has attempted to show by tests the adequacy of injection as compared with serial sections, which are often relied on by those who believe in the local origin of endothelium. He injected small lymphatics, drew their outlines, then sectioned the tissue and reconstructed the vessels. He found that the lines formerly occupied by the continuous vessels were occupied by series of isolated, black, bead-like areas. Doubtless one does well to admit that shrinkage actually takes place in preserved tissues, but doubtless also there is no other case in the literature where the series of isolated spaces comparable to these are individually figured in solid black. If one should combine complete injection with serial sectioning, and find lines of discontinuous injected spaces he would probably not commit the error of regarding them as always having been out of continuity. In all probability the only recorded case of an injected isolated vascular space, besides those figured by Clark, is that of McClure's subocular sac; this was observed in the living condition to be independent, and was injected directly.

As already stated, those who believe endothelium to form entirely by proliferation of a small, restricted yolk-sac anlage have been strengthened in their belief by observation of the sprouting of living endothelium. On the other hand, direct observation of the mesenchymal synthesis of endothelium is by no means wanting. As early as 1885 Wenckebach (77) described with great accuracy the remarkable 'Bewusstsein' with which single mesenchyme cells migrate by amoeboid movement to arrange themselves into endothelial tubes on the teleost yolk-

sac; he also observed the same process in the embryonic body, but here the process was a re-arrangement rather than an extensive migration. So thorough were the observations of Wenckebach that later observations on the living telost yolk-sac by Raffeale (50), Stockard (66), and myself (52, 53) have added little of importance to our knowledge of the process given us by Wenckebach.

III. THE EXPERIMENTAL WORK

The great problem of development is to determine as far as possible, the sequence and origin of differentiations and the extrinsic and intrinsic forces by which they may be modified. Much has already been accomplished in tracing the sequence of morphological differentiations to earlier and earlier stages of development, but relatively little has been learned as to the nature and causes of differentiation itself. By the observation of purely normal processes one sooner or later comes to a place beyond which he can make little if any progress in the study of such problems; but the history of biology in the last twenty-five years has shown that much may be learned by the comparison of normal and abnormal processes, and especially by the combination of observations of normal processes with experiments which may be varied indefinitely.—Conklin.¹

Prior to the year 1913 it might well have been said that the status of the controversy over the origin of endothelium was that in which the observation of the normal process had practically reached its limits, so far as the general acceptance of one view or the other was concerned. Those who believed serial sections to be adequate were not convinced by those who believed in the adequacy of injection, and vice versa. Also, those who had seen the sprouting of living endothelium failed to take into account the evidence at hand that isolated mesenchyme cells had been seen to form endothelium. The question resolved itself into one of methods; that certain definite results could be obtained by each of these diverse methods was no longer questioned by any one. As affirmed by Bartels concerning one of the methods, we might well affirm concerning the questions involved in all the methods employed; they were "philosophical, and not anatomical questions." The account of the develop-

¹ Journal of the Academy of Natural Sciences of Philadelphia, vol. 15, Second Series, 1912, pp. 503-4.

ment of the vascular system as given in Keibel and Mall's *Human Embryology*, 1912, probably represents the consensus of opinion among the majority of American and European anatomists up to 1913—namely that the vascular tissues were of angioblastic origin. A thorough survey of the literature and most recent text books does not lead one to agree with Stockard (66, p. 587) that the recent defenses (Stockard considers them 'revivals') of the ingrowth theory were prompted by purely 'literary reasons.' Also it is improbable that we have reached the era in which philosophical questions can be divorced from the interpretations of the results reached by our methods.

It may well be said that the thirtieth session of the American Association of Anatomists, 1913, marks a new epoch in the endothelium controversy. Schulte at that time showed that the anlagen of the umbilical vein develop locally from parietal mesoderm entirely away from splanchnopleuric vascular anlagen. McClure showed that an isolated lymphatic anlage could be injected directly and independently. But in addition to these significant morphological contributions there stands out in importance the experimental work of Miller and McWhorter (40) who had obtained blood-vessels on the operated sides of chick embryos, one side of which had been excised from the yolk-sac blastoderm. This work is not the first experimental investigation of the problem, but it marks a revival of that sort of investigation of endothelium; it marks the beginning of a serious appreciation of such work, and a concession on the part of many anatomists that the local origin theory is not a priori untenable.

As already stated, the work of Miller and McWhorter was not the first attempt to utilize the experimental method in the solution of problems having to do with the origin of the vascular tissues. In the year 1887 there were three experimental investigations bearing on such problems—the investigations of Budge, Gerlach, and Uskow.

Budge (5) proposed to confirm the view of His by destroying the germinal disc of unincubated eggs, leaving the germ-walls intact so that the latter might demonstrate its independence as the source of the vascular tissue. Gerlach (14) tried to deter-

mine experimentally whether primitive-streak mesoderm or parablast was the source of the vascular tissue; this he tried by preventing the development of the primitive streak. He was unable to obtain decisive results. Uskow (72) likewise attempted to arrest the development of the mesoderm, but was unsuccessful.

The work of Gräper and Hahn along these same lines furnishes a foundation for the subsequent experimental work on vasculogenesis. Their observations have, unfortunately, received little attention from later investigators; for this reason it seems advisable to consider rather minutely their methods and results.

Gräper's work

Gräper's investigation (16) is primarily an investigation of the process of heart-formation, in which the main purpose is to show that the heart, in accordance with the 'Rablsche Erklärung,' is essentially a fused pair of vitelline veins, and its efferent vessels are formed in genetic continuity with its own endothelium. The work attempts to show the explicability of double and multiple hearts on the basis of a failure of the mesial fusion of the endothelial tubes, which originally were laterally located. Evidently Gräper regarded our knowledge of heart formation as somewhat uncertain and theoretical. A second purpose of Gräper's work, which was not essentially experimental, was to prove that the earliest vascular tissues on the yolk-sac are of entodermal origin; that they are formed from entodermal cells which separate from that layer, invade the germ-wall, and emerge to form endothelial cells and blood-cells; that at least some of the invading cells become 'Dotterträger' which participate in the formation of vascular tissues and in their nourishment. The work attempts to disprove the view of Rückert and Mollier that the vascular tissue is derived from primitive-streak mesoderm which is proliferated into or migrates into the vascular area and possibly into the germ-wall.

Ordinarily when blood-islands are first recognizable, they are found lying between the mesoderm and the germ-wall. As Rückert has observed, their position when first recognizable

gives little clue to their ultimate origin. Gräper points out that Rückert's study of avian blood-islands was confined largely to the posterior portion of the blastoderm, where he (Gräper) believes the picture is not clearly marked; his own studies deal mostly with the anterior portion of chick blastoderms in which the head-process has a length of about one millimeter. In this location he was able to find isolated blood-islands in a region not yet invaded by laminated mesoderm. He sometimes found blood-islands more than a millimeter distant from any mesoderm. Such blood-islands lay between germ-wall entoderm and ectoderm. Relying upon the extreme improbability that their origin should ever be regarded as ectodermal, he believes that such blood-islands furnish conclusive evidence for the entodermal origin of the vascular tissues.

Rückert (Hertwig's Handbuch, Bd. 1, S. 1180) writes:

Worin besteht aber die Bedeutung des Dotters für die Blutbildung der Wirbeltiere? Auch hier gibt das Ei des *Platydictylus* einen Fingerzeig. Da die Verdickung des unteren Blattes erst nach dem Erscheinen der Blutanlagen auftritt bezw. (vorn) zu voller Ausbildung gelangt, so kann dieselbe nicht die Aufgabe haben, den Entoblast zur Abgabe von Zellenmaterial an die Blutinsel vorzubereiten. Das Wesen des Vorganges kann also nicht in einer Zellen sondern nur in einer Stoffabgabe des Dotters an die Blutanlagen beruhen.

Gräper dismisses this observation of Rückert's with a statement that it apparently rests "auf Grund vergleichender Forschungen." If it be true, as in the instance cited by Rückert, that blood-islands may develop in a region where there is as yet no thickened entoderm, the isolated blood-islands of Gräper are not all-significant, albeit they display a condition of extreme interest. If blood-islands can develop at a distance from mesoderm, and at a distance from thickened entoderm in another, it is evident that no valid generalization can be made concerning either of these layers as the sole source of the vascular tissue.

Jan Tur (71) described vascular Anlagen completely isolated from entoderm, mesoderm, and from ectoderm. Yet he stated:

"Les éléments de ce 'parablaste accessoire' qu'on pourrait désigner sous le nom de 'parablaste sous-germinale' peuvent donner naissance à de vraies formations vasculaires dont la

valeur est nulle, mais qui prouvent la théorie de l'origine entodermo-parablastique des vaisseaux sanguins." (Parablaste here refers to germ-wall.) If this case reported by Jan Tur be of significance, one might, in following Gräper's line of reasoning, be compelled to enunciate a new germ-layer. Perhaps a most feasible explanation of these isolated blood-islands is the assumption that we are here dealing with migrating mesodermal (mesenchymal) cells; such cells may perhaps arise either from mesoderm, entoderm, or both. Once such a cell has arisen from entoderm or mesoderm, it might well be designated as mesoderm or mesenchyme. As will be considered later, the work of Wenckebach, Raffeale, Stockard, and the writer has shown, the teleost yolk-sac, entirely devoid of true entoderm, furnishes an excellent place in which to study the actual migration of living mesenchyme cells and their transformation into vascular tissue. A study of such migrating cells might convince one that the traversing of a distance of 1 mm. by mesenchyme cells would not be out of the question in case of Gräper's isolated blood-islands.

Rückert points out the possibility that even though the cells emerging from the germ-wall may be closely approximated to the entoderm, they may conceivably have come from mesoderm. Gräper answers this by maintaining that ultimately all the mesoderm in question came from entoderm (which is certainly not true in case of primitive-streak mesoderm in the chick); Gräper also maintains (p. 381) that even if the cells in question had come from mesoderm, they should necessarily be regarded as entoderm when they were in association with that layer, "so könnte man doch unmöglich von mesodermaler Bildungsweise reden." Such confusion at least shows the need of a definite terminology.

van der Stricht (67) described yolk-laden cells in the mesoderm, and believed that such cells had taken on a yolk-content by means of protoplasmic processes which reached down into the yolk. This offers a possible explanation of the "Dotter-träger" of many authors. In some cases such protoplasmic processes might not be preserved in fixed material.

On one point most observers are agreed. Whatever may be the origin of the prevascular cells of the yolk-sac, they leave, perhaps by migration, one or some of the laminated primitive layers before they acquire their vascular characteristics. Such active movement is more characteristic of mesodermal than of entodermal cells.

Prior to its publication, the work of Hahn was known to Gräper. As will be considered later, Hahn attempted to prove that primitive-streak mesoderm is the source of the yolk-sac blood-islands. This he did by destroying the region lateral to the posterior portion of the primitive streak. He obtained embryos whose blastoderms on that side were in most cases entirely devoid of blood-islands. Gräper performed these same experiments, but claimed that it is necessary to destroy not only the existing vascular tissue at the operative stage, but also the germ-wall as well; he claims from this that the germ-wall and not the primitive-streak mesoderm is responsible for the vascular tissue.

The work of Gräper also deals with the origin of intraembryonic vessels. On the operated sides of his experimental chicks he obtained vitelline veins, endocardia and dorsal aortae. In the description of his embryo 'G' he gives the line of reasoning by which he interpreted his results as not supporting the views of Rückert. On p. 396, describing the operated side, he states:

An der Stelle nun, wo man die zweite Herzhälfte bzw. die Vena omphalomesenterica erwartet hätte, sieht man zwischen Entoderm und Mesoderm einen rundlichen Zellhaufen (Z), der ganz den Eindruck einer Blutinsel macht. Verfolgt man diese Masse nach vorn und hinten, so erhält man von Zeit zu Zeit Bilder, die genau mit denen übereinstimmen, die Blutinseln geben, die in der Bildung bluthaltiger Gefässe begriffen sind. Vor der vorderen Darmforte kann man eine Verbindung mit dem 'Herzen' der anderen Seite nachweisen. Es macht also den Eindruck, als ob hier eine autochthone Bildung eines Gefässes stattgehabt hätte. Dies ist auch tatsächlich der Fall, nur nicht im Sinne derer, die behaupten, dass alle grösseren Gefässe in loco entstanden. Es kommt hier vielmehr eine Zufälligkeit in Betracht, die nachzuweisen mir in diesem Falle glücklicherweise gelang. Verfolgt man nämlich die Serie durch eine grössere Anzahl von Schnitten nach hinten, so sieht man diesen Blutinselstrang in ein kleines Divertikel übergehen, das an der Stelle liegt, wo Entoderm und Ectoderm vernarbt

sind. Dieses Divertikel, das Fig. 24 darstellt, enthält noch reichlich in Resorption begriffenden Dotter. Hieraus sehen wir, dass es bei der Operation nicht gelungen war, die Area opaca vollständig zu entfernen, es waren vielmehr noch Keimwallreste central von der Narbe geblieben. Hierzu kommt eine starke Verschmälerung der Area pellucida, wie sie ja stets an den operierten Embryonen beobachtet wurde. Durch diese Umstände war es möglich, dass die Keimwallreste in den Embryo einbezogen wurden. . . . Es bestärkt dieser Embryo also keineswegs die Theorie der lokalen Entstehung der Gefässe in Embryo, sondern ist vielmehr, besonders mit dem Embryo C zusammen, ein Beweis für die Entstehung der Blutinseln aus den Dotterzellen.

In my own experience I have found too that if embryonic tissue be left in communication with an area vasculosa bearing blood-islands the vascular tissues in the two regions invariably become connected.

Gräper also lays great stress on his embryo 'C.' In the interpretation of the conditions in this embryo I believe that Gräper is in error, provided one may judge from the appearance of his figure 27. On p. 398 he describes the operated side as follows:

Die Narbe geht rechts dicht an der Grenze zwischen Area opaca und pellucida hin, so dass der grösste Teil der letzteren erhalten ist. Der hintere Teil des Primitivstreifens wurde vollständig vernichtet, so dass auch am Embryo nur die vordere Hälfte entwickelt ist. . . . Bei näherer Prüfung der Schnitte, bemerkt man links ein schön entwickeltes Herz (Fig. 29), rechts dagegen eine ähnliche Erweiterung, die mit einem Herz nichts gemein hat (Fig. 28). Das Mesoderm spaltet sich hier typisch in seine beiden Blätter. Die Splanchnopleura aber ist hier im Gegensatz zur anderen Seite und zum normalen Verhalten auch an der Stelle, wo die Herzhälfte zu suchen ist, sehr dünn und legt sich dem Entoderm eng an. Zwischen Entoderm und Splanchnopleura sind auf dieser Seite in der ganzen Serie keine einzelnen Zellen oder Zellenzüge zu finden. Hier sei erwähnt, dass es sich aus der Serie leicht feststellen lässt, dass die betreffende Zellschicht wirklich die Somatopleura ist und nicht, wie es den Anschein erwecken könnte, die Wand eines abnorm erweiterten Gefässes. Durch diese glückliche Experiment wird also der oben erwähnte Einwurf, der gemacht werden könnte, entkräftet. Es ist nämlich sehr wohl die Stelle, wo sich das Herz sonst bildet, und darüber hinaus noch ein grosses Stück Area pellucida vorhanden, dennoch ist aber von einer Herzhälfte nichts zu sehen, weil eben die betreffende Seite der Area opaca, aus der, wie wir oben erwähnt haben, das Material für die Gefässbildung stammt, durch die Narbe vollständig abgetrennt ist.

Dieser Embryo scheint mir besonders die Ansicht Rückerts zu entkräften, denn obgleich die ganze hintere Hälfte des Primitivstreif-

ens in jungem Stadium vernichtet war, haben sich doch auf der Seite, wo die Area opaca unversehrt blieb, Gefäße und ein Herz gebildet, was nicht hätte geschehen dürfen, wenn jene Ansicht, auf der die Hahn-schen Experimente basieren, richtig wäre.

Judging from his figure 27, one may well question whether the 'Narbe' has really excluded the area opaca. A line of separation within the area pellucida has never in my experience yielded such an enormously thick 'Narbe' as that shown in this figure. The tissue on the extreme right of the figure is in all probability a portion of the area opaca. Its failure to develop endothelial cavities (and perhaps to absorb yolk) is difficult to explain. One never finds the splanchnopleuric mesoderm (myocardium) in the heart region a single-layered structure unless there is a pronounced oedema, so that the distension produced by the coelomic fluid enormously stretches the mesodermal walls. This distension may furnish a mechanical explanation of the failure of the cardiac endothelium to form on the side on which the oedematous condition is exhibited. Certain it is that endocardial tissue had little space in which to develop under such conditions. The space between splanchnopleure and entoderm is completely occluded; in other words, if this explanation be correct, endothelium was potentially able to develop if the mechanical relations of the coelom had not prevented it. One could also assume that those conditions which produced the abnormal coelom had likewise injured the tissue which would have produced prevascular Anlagen, and that both abnormal conditions resulted from the same cause.

Hahn (17, pp. 410-413) has also commented on the conditions in Gräper's embryo 'C'. He points out the fact that in "Gräper's embryo 'A' the heart is also unilateral. He states (p. 410):

Die Tatsache, dass eine der operierten Seite entsprechende Herzhälfte bei Embryo A nicht zur Entwicklung gelangt ist, begründet nun Gräper damit, dass dieselbe 'eben nicht nötig war.' Er führt dies dann noch weiter aus mit den Worten: 'Auf der operierten Seite wurde die Anlage der Gefäße durch den Eingriff verzögert (also nicht total verhindert!), so dass die andre Seite Zeit hatte, eine Herzhälfte zu bilden, die dem ganzen Herzen gleichwertig war; numehr war die Bildung des zweiten Herzens unnötig und unterblieb.'

Hahn calls attention to the fact that if such argument as that of Gräper concerning embryo 'A' were valid it could likewise apply to the conditions in Gräper's embryo 'C,' which is above described. I believe with Hahn that the question of necessity is inapplicable equally in both cases. In both these instances the development of endothelium was 'unmöglich' whether it was 'unnötig' or not. Gräper's explanation of his embryo 'A' would defeat his own theory of multiple hearts, in that only one is 'nötig;' it is inconceivable that all supernumerary heart-anlagen have equal advantages for their own preservation and development. Doubtless in both Gräper's embryos 'A' and 'C' he has simply produced conditions such that one-half the heart anlage was unable to develop—otherwise both would have developed bilateral hearts if the embryos were normal prior to operation. If an endocardial anlage ever started on the operated side in either case, it could in all probability have been detected in these embryos when they were sectioned.

Gräper misinterprets Hahn's conclusions when he states that the development of endothelium in his own embryo 'C' disproves the validity of Hahn's work. So far as I am aware, Hahn did not claim that a destruction of the posterior portion of the primitive streak would inhibit the formation of endothelium.

Gräper's operations were generally performed on such early stages that the orientation of the embryo was uncertain. In some cases his 'posterior' cauterizations were no doubt somewhat lateral.

I am convinced from my own experiments at least, that Gräper's one 'fortunate case,' embryo 'C,' is insufficient to overthrow the view of Rückert and Hahn.

2. The work of Hahn, and Miller and McWhorter

As we have already seen, the work of Hahn was announced by Rückert in Hertwig's *Handbuch* before Gräper's account was published, but the latter account was published prior to the publication of Hahn's own observations; owing to these circumstances the work of each was known to the other. Their results

were quite different; this explains the fact that it is impossible to review each work separately.

Hahn's work (17) covers a number of years of experimentation. His descriptions are concerned with the conditions obtained in seven blastoderms. Like Gräper, he employed the cauterization-method in separating the embryonic body on one side from the blastoderm, and in the destroyal of the primitive-streak mesoderm. In most cases he found that a destruction of the posterior portion of a side of the primitive streak prevented the formation of blood-islands in the blastoderm on that side. In one case (Embryo III, p. 376) he describes a large vesicle on the operated side of the blastoderm, lying near the wall of the wound opposite the embryonic body. This vesicle is filled with fluid and the cells of its lining are flattened out so as to resemble endothelium. He states:

Es ist nun eine Frage, ob man solche von Flüssigkeit erfüllte, jedoch nie geformte Elemente enthaltende sinus als Gefässanlagen, oder als cytöse Bildungen, die mit typischen Vorgängen nichts zu tun haben, auffassen will. Jedenfalls scheint ihre Bildungsweise mir auf die Fähigkeit des Dötterendoblast hinzuweisen, leere mit teilweiser Endothelauskleidung versehene Lacunen bilden zu können.

Lying below this vesicle was a small isolated group of blood cells, some of which were intimately associated with the lower wall of the vesicle, but none entered it. Hahn considers this case as possibly analogous to some of the "‘aberranten Blutinseln’ Gräpers." He states that the emptiness of the vesicle of corpuscles "spricht nicht gerade für ihre Natur als Blutanlagen," but leaves the question open.

Concerning the origin of the intraembryonic vessels, Hahn's results speak exclusively for a local mesodermal origin. On page 426 he states:

Wenn ich demgemäss meine eignen Beobachtungen zusammenfasse, so komme ich zu demselben Schlusse wie bezüglich der Herkunft der Aortenzellen, dass nämlich das Material für das Herzenendothel ebenso wie das Aorta 'genetisch unabhängig' ist sowohl von Dötterendoblast wie von dem embryonalen Teil des Darmdrüsenblattes. Die Relation zwischen dem Auftreten einer Herzanlage auf der gestörten Seite einerseits und dem Umfang des erhaltenen Splanchnopleura-

stückes andererseits, ferner die Beobachtung des Austritts des fraglichen Materials aus diesem Rest des visceralen Mesoblastblattes zwingen mich, eine lokale Entstehung der Endothelelements aus der genannten Quelle zu vertreten.

Two of his experiments are of particular interest. In his embryo I, the posterior end of the dorsal aorta is described as follows:

Im Bereiche des letzten Ursegments betheilen sich nur mehr ein bis zwei Endothelzellen auf dem Querschnitt an der Bildung der Aortenwand, und fast gleichzeitig mit dem Aufhören des letzten Restes einer Seitenplatte ist auch kurz nach dem Eintritt in die unsegmentierte Rumpfregeion die Aorta verschwunden. Erst nach einer Fläche der auf der linken Körperseite restierenden mittleren Keimblattmasse neuerdings erst einzelne, dann zwei bis drei Gefäßzellen am Schnitt wieder auf, die auseinanderweichend wiederum ein deutliches Gefäßrohr umgrenzen (Fig. 24, Taf. XII).

In his embryo II the posterior locus of origin of the vitelline vein and cardiac endothelium of the operated side is described (p. 402) as follows:

Diese besteht in einer lokalisierten Auflockerung des hohen mehrzeiligen Epithels des Darmfaserblattes, wodurch es einer Anzahl auffallend spindelförmig ausgezogener Elemente möglich wird, theils isoliert, theils in kleinen Ketten aus dem Epithelverbande herauszutreten und mehr und mehr frei werden. Diese lagern sich in dem Spaltraum zwischen Darmendoblast und visceralen Mesoblast herein und bleiben dabei zunächst mit ihrer Ursprungsstätte noch in Zusammenhang.

Farther anteriorly a lumen appears in the group of cells which may be traced into the heart. The cardiac tube has no connection with that of the normal side. Anteriorly (p. 404):

. . . . es verengt sich rasch und löst sich in einzelne lateralwärts ziehende Gefässanlagen auf, ohne dass, wie früher erwähnt, es gelingt ein aufsteigendes Bogengefäß und seine Einmündung in die dorsale Aorta zu finden.

Hahn evidently anticipated the possible objection that the normal endothelium might have vascularized the operated side. He gives reasons for believing that such was not the case.

The experiments of Miller and McWhorter (39), like those of Hahn, consisted in the separation of one side (and also one end)

of chick embryos from the extraembryonic blastoderm. The conditions which they obtained were very similar to the results of Hahn. Their work contributes two distinct advances over that of Hahn. They reconstructed the endothelial tubes obtained and still more important, their method of separation of the axial portion of the body from the blastoderm was by incision rather than by the crude and uncontrollable method of cauterization or burning. The amount of injury along the line of separation is certainly much smaller in the method introduced by Miller and McWhorter. Their work was submitted as proof of the local origin of endothelium. Prior to, and following its publication the objections urged against this work were inclusive of the following: (1) the incisions may not have been made sufficiently close to the embryonic axis; (2) they may not have been made sufficiently early; (3) endothelium may have grown in from the opposite side or from the ends. Prior to my own work (52) the only published comment on the interpretation of local origin in such experimental cases, besides a short discussion by Schulte is that of Bremer (4, p. 462) who in discussing the work of Gräper, Hahn, Miller and McWhorter, states:

From the differences in the conclusions reached by two of these authors (Gräper and Hahn) it seems certain that more work should be done along these lines before a consensus of opinion can be expected. I wish to point out a few possibilities which should, I think, be considered in any such future work.

As shown in my reconstructions of rabbit embryos, the vascular net has an irregular mesial border, certain strands lying further toward the midline than the position of the future aorta.

Though in young embryos the extension of these strands across the median line of the embryo proper to form a net on the opposite side is rendered impossible by the close approximation of the medullary groove, notochord, and entoderm, yet long before the stage figured in many instances cited by these authors the mesoderm has grown across the median line and might afford a pathway for endothelial sprouts from side to side.

Another and earlier pathway is at the posterior end of the embryo, behind the primitive streak where the mesoderm very early extends across the median line. The angioblast cords, by which connections from recognizable blood-vessels to apparently isolated angiocysts can be traced (if we accept, for the moment, and for the purpose of argument, the extension theory) are delicate strands, easily overlooked, and

moreover may last only a few hours, if the mechanical conditions are not favorable to their continued development into vessels. It is not to be expected, therefore, that anything short of a very complete series of such operated embryos, fixed at progressively longer intervals of incubation after operation, can settle whether or not there is any extension from the opposite side.

Concerning the communication of vessels of the two sides, I may say that such connections may sometimes be found in the head-region ventral to the pharynx in completely isolated head-meroplasts. Such cases (fig. 22) probably have little significance for the experiments of Hahn, and Miller and McWhorter.

Whatever may have been the validity of the objections urged against these experimental results, the above review gives the exact status of the endothelium-controversy at the time at which the present work was begun. In general my results have been made known through a previous publication (52). These former results, together with many more recent ones are incorporated in the present communication.

IV. MATERIAL, METHODS AND OBSERVATIONS

1. *Chick material and mechanical methods*

We have considered the previous experimental results on chick material, and have enumerated the objections urged against their finality. Doubtless the most serious objection to the experimental work on chick material which had preceded my own, was that of the possibility of ingrowth from the opposite side. It had been suggested that nothing short of a study of a large number of closely graded stages could furnish a basis for conclusive evidence. It is evident, however, that such a study might still remain open to the objection of those who require still more thorough-going investigations. Such a situation must necessarily confront us unless there be some means of altering the technique of operation, or increasing its severity to an extent which will eliminate the necessity (though by no means, the desirability) of such an extensive study. It occurred to the writer that a feasible method of eliminating the possibility both of ingrowth and of further extension from the oppo-

site side, would be that of isolating both sides from the outlying blastoderm—or better still, to isolate completely the embryonic body or portions of it from all the outlying blastoderm at a time before the embryonic tissue is vascularized. It is evident that if endothelium should then appear in the isolated tissue following further incubation, such endothelium could not possibly represent an extension or ingrowth of a yolk-sac vessel. Under these conditions the question of growth from the opposite side would not concern us, so far as the proof of local origin is involved.

As before noted, it was objected that the operations of Miller and McWhorter may not have been done early enough, and that their incisions may not have been made sufficiently close to the embryonic tissue. In seeking a means of overcoming these objections I happened on the following procedure: at the time of operation it is possible to remove the yolk-sac blastoderm lateral to the line of separation on one or both sides. In case only a head-fragment is to be further incubated, it is possible to remove with the blastoderm that part of the body-axis which normally should first contain blood-vessels. This tissue is preserved at once and sectioned. In this manner one can determine exactly the status of the extraembryonic vascular tissues. By this means it is possible, with safety, to let the embryos develop to a much more advanced stage before operation than would be permissible by the methods of any previous observers. From this it is not to be inferred that in all cases it is desirable to wait until the latest possible time of exclusion of ingrowth before performing the operations. If the general method be trustworthy, one should be able to get positive results long before vascular tissue has developed dangerously near the embryo's body. The great advantage of operating at as late a stage as possible is, that the abnormality produced is likely to be less, and growth and differentiation proceed farther the older the embryo is at the time of operation. If allowed to summarize what I believe to be my contributions to the experimental technique of studying endothelium, I would say that so far as mechanical methods are concerned they are these: com-

plete isolation of the unvascularized tissue, and a means of obtaining accurate knowledge of the conditions in the outlying tissue. Given these, the method becomes a critical one. It should satisfy all reasonable demands of experimental proof. To say the very least, the endothelium which can develop in the isolated tissue under such conditions cannot have grown in from an outside source.

Whether the endothelium which undoubtedly does form under these conditions represents in a faithful manner the essentials of its normal development is not for me to say. All that can be claimed is that my figures show conditions which were actually obtained. Further study alone can determine what significance is to be attached to these conditions.

Record of material used in illustrations in Part I

TYPE	NO.	INTERSO- MITIC GROOVES AT TIME OF OPERATION	TOTAL IN- CUBATION	STAIN	BLASTO- DERM EX- AMINED	ILLUSTRATED IN FIGURES
			<i>hours</i>			
I	19	1	32	m.b.-e.	+	11
I	24	0	29	m.b.-e.	+	12
I	18	1	30	m.b.-e.	+	13
II	3	2	48	m.b.-e.	++	16, 17, 18
I	31	2	32	m.b.-e.	++	14, 19, 21
I	17	1	33	m.b.-e.	+	15
I	122	0	34	m.b.-e.	++	20, 22
I	111	0	31	m.b.-e.	—	29
I	34	0	29	m.b.-e.	—	35, 36
II	133	0	38	Fe.h.	+	23
II	96	3	48	Fe.h.	—	24
II	116	1	40	Fe.h.	+	25, 26, 27
II	52	1	38	Fe.h.	+	28
II	124	2	41	Fe.h.	+	30
II	149	0	36	Fe.h.	+	31, 32, 33
II	53	2	39	Fe.h.	+	34
II	88	2	44	Bx.c.	+	37, 38
II	14	0	35	Fe.h.	+	39, 40, 41, 42, 43,
11	48	0	37	Fe.h.	+	44, 45, 46, 47, 48

Explanation of abbreviations used in table: *m.b.-e.* refers to my modification of Mann's methyl blue-eosin stain, *Fe.h.* indicates iron haematoxylin; *Bx.c.* indicates borax carmine. Two plus-marks indicate that the extraembryonic blastoderm is shown in illustration.

b. Complete isolation of meroplasts. In general the methods of operation have fallen under two types: Experiments designated as Type I are those in which a small portion of the embryonic body was completely isolated from the outlying blastoderm. An examination of figures 1 and 2 will explain the methods by which this complete separation was accomplished. Chick embryos prior to the formation of intersomitic grooves, and stages up to the formation of three intersomitic grooves constitute the

1 Diagram to illustrate the blastodermal incisions in experiments of Types I and II. Broken lines represent the incisions in Type I. Dotted line *G* to *H* indicates the transverse incision in Type II in which incision *E* to *F* is omitted.

2 Lateral view of the head-fragment of Type I pushed back to be severed from the proamnion at point where a broken line intersects.

material studied. It was found, on examination of the blastoderms that blood vessels were not generally to be found near the embryonic axis at the time of the first intersomitic groove. There are, however, sufficient exceptions to this to make an examination of the blastoderms always desirable. Normal incubation was generally allowed for about eighteen to twenty hours; this had to be varied in different seasons.

The Type I operation consisted in this: longitudinal incisions were first made, separating laterally the anterior region of the

embryonic axis (fig. 1, *A-B* and *C-D*). Next, a transverse incision (*E-F*) served to sever the anterior portion of the body from the more posterior portion; this incision was usually made at the anterior intestinal portal. At this time, before completely separating the head-fragment from the small band of tissue which held it anteriorly, it was found advisable to remove the main portion of the blastoderm. The method of doing this will be understood by following the broken curved lines from points *B* and *D*, laterally and posteriorly; a transverse incision was then made, joining their posterior extremities. The large piece of blastoderm obtained by this means was preserved at once, to be sectioned later. The head-fragment was then completely isolated by the incision shown in figure 2. This figure represents a side view of the head (represented entirely too large) which has been pushed back so that the incision can be made with greater ease. Such a fragment generally sinks to the bottom of the sub-germinal cavity. Following the operation the egg was sealed and incubated to a total age of from twenty-four to forty-eight hours; after that time, degenerative changes could be seen in the tissues. Differentiation did not usually proceed beyond that of a normal thirty-two hour embryo. Growth, on the other hand, varied greatly. Meroplasts equally differentiated might vary greatly in size. As I have previously noted (52, p. 334) it seems that the embryonic meroplast "possesses an inherent capacity for differentiation which tides it over to a time when heart-pulsation should normally provide a means of tissue-respiration."

After the final incubation the tissue-fragment was removed and preserved in picro-acetic acid, sectioned, and stained. The stain ordinarily employed was my modification (51) of Mann's methyl blue-eosin stain. In a number of cases in which an attempt was made to utilize mitotic figures as a means of determining the origin of the prevascular tissue, the sections were stained in iron-hematoxylin and counterstained with acidulated methyl blue. Ordinarily the sections were cut 4 micra in thickness.

Sections of such fragments displayed surprisingly normal conditions. The neural grooves would often close normally. If the incision *E-F* (fig. 1) were made posterior to the anterior intestinal portal, the flat blastoderm would often conalesce to form a tube. The sections often contained coelomic cavities apparently farther anteriorly than one should expect to find in the normal embryo. Above all, such fragments generally contained blood-vessels in various stages of development.

A very typical picture, frequently obtained in head-meroplasts severed at the anterior intestinal portal is that seen in figure 11. Here in the fore-brain region the neural folds have failed to meet each other. A head-coelom has developed. On the right side of the figure there is a rather large endothelial cavity with a smaller one below it. Such structures are absent on the opposite side in this particular plane of section.

Since some of the figures of this paper have been previously described (52) they need not be dwelt on at great length. Without giving a great deal of evidence concerning the origin of the earliest prevascular cells, I stated (52, p. 334) that "between the base of the coelomic 'pouch' and the pharyngeal entoderm, rounded or cuboidal cells become proliferated. Their point of origin is in most cases between the base of the coelomic 'pouch' and the pharyngeal entoderm where it is difficult to determine which of these two epithelia is of primary importance in such cell-proliferation." The cells in question are shown in figure 12. In figure 13, it will be seen that ventral and lateral to the pharynx such cells have arranged themselves into a parenchymatous complex which merges dorsally into the indifferent mesenchyme. At the time when this figure was first published, I was inclined to believe that such a condition represented an essential stage in the formation of endothelium in this region, and hinted that the resolution of such a complex into endothelial tubes might be closely related to the accumulation of plasma in its meshes. My more recent experiments lead me to believe that such a condition may not necessarily be passed through by the tissue in this region; that the tubes may be formed by a more direct and active manner, in which isolated vesicles are

formed without the intervention of a parenchymatous stage; that such isolated vesicles coalesce to form the tubes; that such tubes are enlarged by the addition of single mesenchyme cells, or by the addition of solid or hollow groups of cells. There appear certain cases, however, exhibiting a parenchymatous arrangement of cells which, from their staining reaction, are to be interpreted as true endothelial cells.

In figure 14 is shown a cross (perhaps frontally inclined) section of a head-meroplast. On the right the incision was very close to the brain tube. The dorsal mesenchyme has arranged itself into the form of an epithelium. The incision included the pharynx, causing it to gap open and be displaced by tension of the opposite side. The cut edges of ectoderm and entoderm end blindly. Lateral to the ventrally directed U-shaped portion of the pharynx an endothelial tube is seen on each side. Between these two tubes is a solid ventral connection. The conditions here resemble heart-formation to a certain extent. The coelomic walls have met mesially, but their place of meeting is ventral to the pharynx; the latter has not been constricted for the reason that it was already tubular at the time at which the operation was performed, whereas in heart-formation the transformation of flat entoderm into tubular pharynx is an essential process. A more accurate figure of the ventral region of a neighboring section is shown in figure 21, in which many structures are figured less diagrammatically.

Conditions somewhat similar to these are shown in figure 15. The longitudinal incision, as seen on the left of the figure, was not far from the median plane, and here again the mesenchyme has formed an epithelium which merges so gradually into ectoderm that it is impossible to determine where that layer begins. It will be noted that on the left side of this embryo no endothelium has formed. When the incision is made so close to the median plane, such conditions often result; it seems to stimulate a vigorous growth of mesenchyme which becomes so dense that the formation of endothelium is impossible. Such close incisions are not necessary for the prevention of vascular ingrowth.

A condition similar to those described in embryo Type I, No. 31 (figs. 14 and 21) is seen in Type I, No. 122 (fig. 22). In this embryo the endothelial tubes have a median connection, but in this section their cavities are distinct. Posteriorly the cavities themselves communicate. Despite the great differences in the development of the blastoderms at the time of operation, Type I, No. 31 and Type I, No. 122 show very great similarity of structure; the conditions in the former are shown in figure 19. This figure is a photograph of a cross-section of the blastoderm (exclusive of the meroplast) which was removed at the time of operation. In this operation the incisions *A-B* and *C-D* extended somewhat posterior to the line *E-F*. The plane of section lies between the line *E-F* and an imaginary line *A-C* (fig. 1). Thus we obtain a section showing the body axis, discontinuities corresponding to the longitudinal incisions *A-B* and *C-D*, and lateral to these, the outlying blastoderm. Such a section records both the position of the longitudinal incisions and the conditions of vascular development in the body axis as well as the extra-embryonic area. This section shows the greatest mesial extent of the yolk-sac vascular tissues which are in continuity (fig. 19). Lying here and there, mesial to these vessels, are occasional cells or groups of cells, apparently in process of separating from the splanchnopleure. These may well be regarded as forerunners of vascular tissue. No vessel fulfilling the requirements of a sprout of 'angioblast' had yet approached the median axis. The tissue between the two incisions (fig. 19) represents the middle or anterior part of the hind-brain region. It would perhaps have been the first part of the body to have been 'invaded' by vitelline capillaries, if invasion had been the true process of vascularization. The longitudinal incision represented on the right side of figure 20 was not parallel to the median axis, but approached it anteriorly.

Figure 20 is a photograph of a transverse section of the blastoderm belonging to the meroplast of which figure 22 is a section. The plane of figure 20 is only a short distance behind the line *E-F* of figure 1. The points *A* and *C* in this case lie in the line *E-F*; hence the effects of the longitudinal incisions are not

shown in this section. The stage of operation in this case was considerably before the time of appearance of intersomitic grooves. There is as yet no formation of vascular tissues, even in the area opaca. The mesoderm is still uncleft. This and the foregoing case illustrate very well the advantages of removing the outlying blastoderm at the time of operation. One could, with impunity, have allowed this embryo (Type I, No. 122) to incubate longer, provided the blastoderm had been removed to check the result. I have in no case offered as proof, however, any material in which the vascular tissue of the yolk-sac was seen to have come near the line of longitudinal incision.

Figures 29, 35, and 36 are also from completely isolated fragments, illustrating in a positive manner the local formation of endothelium. But in addition to this they display certain special features of interest in other connections to be dealt with later in this work.

c. Incomplete isolation of meroplasts. Another type of experiment performed was one which may be designated as Type II. In such experiments the operated tissue was not completely severed from the outlying blastoderm. Generally the embryonic tissue left to further incubation consisted of a portion of the body axis united with the opaque area anteriorly by a small strip of proamnion. It was repeatedly observed that any connection with the blastoderm greatly favored the vitality of the meroplast. Again a consultation of figure 1 will make clear the method of operation. Longitudinal incisions corresponding to *A-B* and *C-D* were made of lengths varying from those which separated only a portion of the head, to those which extended back well into the posterior region. In case only the head or trunk-region was desired, an incision *G-H* served to separate the meroplast posteriorly, and also to separate the posterior blastoderm from the more anterior portion of the body-axis. The blastoderm not included in the meroplast was removed at the time of operation to be sectioned.

In meroplast No. 3 of Type II, the longitudinal incisions reached posteriorly to the plane of the line *G-H*, which also shows approximately the position of the transverse incision

which severed the anterior and posterior portions of the embryonic body, and which also was the transverse line of separation of the blastoderm. In this experiment all the blastoderm anterior to the line *G-H* was left for further incubation, while that posterior to it was removed. Figures 16, 17, and 18 show the conditions as seen in sections of the meroplast and its blastoderm-control. Figure 16 is a cross-section through the anterior portion of the fore-brain. The cut edges of the entodermal and ectodermal layers of the proamnion have fused, forming posteriorly a blind sac into which there seems to have grown from the anteriorly lying coelom, a sac which likewise ends blindly within the posterior region of the proamniotic sac. Formerly I regarded the presence of this coelomic sac as abnormal. It is probably a normal structure, so far as the presence of coelom in the proamnion at this stage is concerned. It will be seen that the cut edges of ectoderm and entoderm of the outlying blastoderm have fused. The only possible path by which yolk-sac vessels could have reached the embryonic axis would have been by way of the proamniotic sac. It will be noted that the latter is devoid of blood-vessels.

Figure 17 is a section through the mid-brain region of this same meroplast. Its interpretation will be facilitated by reference again to figure 1. Since the incision *G-H* was made considerably behind the anterior intestinal portal, the axial tissue included between the longitudinal incisions and lying between the anterior intestinal portal and the incision *G-H* was necessarily composed of three layers at the time of operation. Dorsally there was ectoderm, ventrally entoderm, and between the two mesoderm was present. The cut edges of ectoderm and entoderm have fused, and the dorso-ventral expanse of the contained tissue has rounded the axial portion into an almost cylindrical shape. Thus the meroplast has the superficial appearance of a tubular head-portion of the body axis as one finds in the fore-brain region projecting over the blastoderm. Since this section lies behind the anterior intestinal portal, there is no ventrally lying proamniotic sac. Although the body appears to be tubular, it contains no alimentary tube. The ven-

tral wall of the meroplast is really the dorsal wall of the pharynx. The anterior axial tissue is very degenerate in appearance. At about the beginning of the mid-brain region there is an extraordinarily abrupt change in the tissue to a very perfectly normal and healthy condition. This experiment yielded the most satisfactory result which I have been able to obtain, due to some favorable condition which I have never been able to duplicate. Two very well developed aortae are present. Figure 18 is a section through the blastoderm of this same embryo. Its plane passes through the hind-brain region where the first axial vessels should be expected. It shows the freedom of the area pellucida from endothelium.

d. Splanchnopleural concrescence and the mechanics of heart-formation. So far we have considered the conditions in the head region where a portion, at least, of the body axis was tubular at the time of operation. It is evident that the region of the body which becomes tubular by the folding and constriction of flat layers must have some interesting experimental possibilities. In the trunk-region the nearness of the longitudinal incisions to the median line profoundly affects the reaction which the tissue will give. If the incisions be relatively near that line the three germ-layers retain their horizontal position, or at least show no tendency towards concrescence (fig. 31; the right sides of figs. 24 and 44). If the incisions be made slightly more lateral (fig. 39) there is a rather abortive attempt at concrescence to produce an alimentary tract. One side will exhibit this tendency, even though the incision on the opposite side was so close to the body-axis that there could be no concrescence (figs. 24, 25, 35, and 44). This shows conclusively that the process is not one of mutual attraction of the opposite sides. Figure 23 shows in a most striking manner the close relation existing between the nearness of incision to the body axis and the degree of concrescence. In this instance the incisions are unequally distant from the median line, and the side containing the greater amount of tissue underwent the greater amount of concrescence. There is almost mathematical exactness in this relation of the amount of intact tissue to the angle of concres-

cence. Incidentally it might be mentioned here, as will be considered later, that the size of the endothelial tubes obtained (fig. 23) also bears a close relation to the amount of tissue left intact. It will be noted (figs. 23, 25, 30, 38, 39, and 44) that there is a tendency for ectoderm, both layers of mesoderm, and entoderm to remain united at the line of incision. When this happens, as it ordinarily does, the parietal mesoderm and the ectoderm share in this ventrally directed concrescence. Thus in some cases the tubular meroplast produced by the fusion of these ventrally directed complexes may be entirely surrounded by ectoderm (fig. 28) or partly so, as in figure 27, in which a part of the ventral 'body wall' is composed of entoderm. In this latter connection it is profitable to consider again the conditions in figure 17 where, as we have already seen, the entire ventral 'body wall' is entodermal. On the right side of this figure it will be noted that the incision was relatively close to the body axis. Whatever attempt there may have been made on this side at a ventral concrescence was annulled by the remarkable dorso-ventral expansion of the tissues, which even caused the entoderm to bend upwards. On the left of the figure it will be seen that the incision was located somewhat more laterally—obviously more lateral than the most lateral extent of the potential dorsal wall of the pharynx. The apex of the projecting fold on the left side represents the point of fusion of ectoderm and entoderm. This fold is somewhat ventrally directed, so that here we have a feeble attempt at a ventral concrescence. It would undoubtedly be possible to get a completely graded series of trunk-meroplasts from those whose entire ventral wall is entodermal, to those entirely surrounded by ectoderm.

It does not always happen that parietal and visceral mesoderm remain united, as one sees in figure 35; but in all cases this parietal complex tends to be somewhat ventrally directed. In no instance have the parietal layers folded dorsally to simulate amnion-formation. It may be that the mechanical conditions of the operation cause the mesodermal layers to be united for a time, and that when they part, the upper complex is unable

to continue a dorsal up-folding. In some cases the parietal layers remain in a practically horizontal position appearing as rather inconspicuous appendages of the visceral lamellae which have evidently continued their growth. This size-difference is shown somewhat in figure 35.

Let us now consider the behavior of the locally formed endothelial tubes during this process. Since in all cases considered the operations were performed at a time before the embryonic fundament was vascularized (as was in most cases actually verified by an examination of the blastoderm themselves) the endothelial tubes in the trunk-region are local formations. Since these endothelial tubes lie in the region of normal heart-formation, their behavior is of interest.

In figure 23, morphologically ventral and internal to the line of incision on each side will be seen in section an endothelial tube. That on the right is the larger, as has already been noted. The adjacent splanchnopleure has invaginated so as to conform to the size and curvature of the surface of the endothelial tube on each side. The conditions remind one of mammalian heart-formation prior to the ventral approach of the bilateral anlagen. It is impossible to say just how far the process would have continued if the tissue had been left to further incubation.

The trunk-meroplast Type II, No. 116 is of interest in that it portrays the process of concrescence as it may be followed progressively from the posterior to the anterior region. Fortunately the incision on the left side (figs. 25 and 26) was nearer to the median line in the posterior region than it was in the anterior region; thus in the posterior region the tissue remaining intact on the left side is much smaller in amount than on the right. As the line of incision on the left side gradually diverged from the median axis anteriorly, the tissue on the two sides became equal by the time the incisions reached as far anteriorly as the tubular head-region. On the left sides of figures 25 and 26 it is seen that the formation of endothelium has been inhibited in the posterior region. Farther anteriorly, however, endothelium has formed on this side so that the median heart

in figure 27 is a bilateral structure. Figure 25 shows a well-advanced concrescence. Entoderm can readily be distinguished from ectoderm by the darkly staining globules. In figure 26 the entodermal layers have met and fused. The entoderm which is to form a portion of the ventral 'body wall' of the meroplast (morphologically the entoderm which lies next the yolk) has severed its connection with the tubular pharyngeal entoderm; as there are no endothelial tubes in the immediate region, the opposed splanchnopleuric mesodermal layers have come directly together. Such endothelium as there is lies ventral to their point of contact. Mechanical conditions may account for the failure of endothelium to form between visceral mesoderm and entoderm at a level dorsal to this place of contact. More anteriorly, in sections not shown in these figures, bilateral endothelial tubes fuse mesially to form a single chambered heart (fig. 27). Well-developed dorsal aortae are present. The thickened entoderm on the ventral side of figure 26 is not material derived from the area vasculosa. Its thickness has been contributed to by the vertically lying fused entoderm which has subsided in consequence of the severing of connection of the latter with the tubular pharynx. The place of incision was within the limits of the area pellucida. Also the entoderm from each side was somewhat thickened as this region contains the 'Narbe' caused by each incision. These conditions are not comparable to those of Gräper's figure 24 (16). In my figure 27 the meroplast has a complete ventral 'body wall' which is composed partly of entoderm. In some sections posterior to this, the duplex nature of the heart is indicated by the duplicity of the endocardium; here it is single. One can here detect the double nature of the myocardium by the small, median, ventrally directed point of fusion of the originally right and left splanchnopleural anlagen. Opposite, close to, and directed towards this myocardial protuberance is a sharp projection of the horizontal splanchnic mesoderm (i.e., the mesoderm associated with the 'body wall' entoderm). These two projections represent sectioned folds which are remnants of the ventral mesentery of the heart; they have been continuous structures but have

lost connection just as in normal heart formation. The part of the coneresced entoderm not utilized in the formation of the pharynx has contributed to the formation of the abnormal 'body wall.' So far as the body wall is concerned, the conditions in this experiment are as if one should remove the yolk of a telolecithal ovum and unite the cut edges of the ectoderm by a splice of splanchnopleure.

Figure 24 is a section through a trunk-meroplast. The incision on the right was rather close to the embryonic axis. On the opposite side the tissue was left in communication with the blastoderm. Conrescence from the left side has not only reached, but has crossed the median line. The large heart is really unilateral, exhibiting conditions closely resembling Gräper's figure 21. This tendency of the unilateral heart to assume the dimensions of a normal heart is often very striking in embryos in which one side is left in communication with the blastoderm.

e. A possible interpretation of certain 'mesothelial funnels' and multiple hearts. One of the most striking phenomena to be observed in meroplasts containing the heart-region is the reaction of visceral mesoderm to the development of endothelial tubes. One is impressed by the fact that where the vitelline veins enter the body axis the visceral mesoderm is profoundly affected. A most interesting problem is the determination of the causal relationships involved. At present it is possible to offer no more than a suggestion for the solution of this problem. This much is certain: in the heart region of these meroplasts excised on each side from the blastoderm, groups of cells become proliferated between entoderm and splanchnic mesoderm; these cells have a tendency to align themselves into solid cords or hollow tubes, sometimes reticular or branched, sometimes singly and sometimes separately as independent structures; correlated with the presence of these structures there is a marked tendency of the adjacent splanchnic mesoderm to attempt to enfold them. Thus if a cord or tube lie longitudinally along the pharyngeal wall, there is a tendency for the adjacent visceral mesoderm to extend a longitudinal fold dorsal to the cord or tube, and a

longitudinal fold ventral to it, so that in section there appears a funnel-shaped reflection of the splanchnic mesoderm towards the dorsal surface of the endothelial tube, and a similar one ventral to it. In case there be more than one endothelial tube, one 'funnel' may project between two tubes (figs. 28 and 29). It is rare indeed to find the apex of such a 'funnel' abutting against or pointing towards the middle convexity of the surface of such a tube. These mesodermal folds have a tendency to project into a plane mesial to the endothelial tube to meet each other, thus completely engulfing the endothelial tube (fig. 3). The internal dorso-mesial element of the upper 'funnel' may fuse with the internal ventro-mesial element of the lower 'funnel;' at the same time the external ventro-lateral element of the dorsal 'funnel' may fuse with the external dorso-lateral element of the ventral 'funnel.' When these fusions have taken place (fig. 43) the process may be carried still further. The layer of mesoderm engulfing the tube may separate from the remaining mesoderm at the raphè of fusion of the apices of the two mesial folds or 'funnels,' so that the endothelial tube is now (fig. 4) completely surrounded by a cylinder of mesoderm which is in every way comparable to a myocardium except that there is neither a dorsal nor a ventral mesentery. What corresponds to one lateral half of the normal dorsal and ventral mesenteries now lies mesial to the unilateral heart as a sheet of mesoderm lying parallel to and closely against the entoderm; anteriorly and posteriorly this mesial surface of the unilateral cardiac tube necessarily joins this layer of mesoderm if the cardiac tube again re-enters the body-axis (fig. 4); also the remaining surface of the myocardium is reflected back as parietal mesoderm.

The preceding explanation applies to the condition in which the unilateral myocardial tube 'leaves' the pericardial cavity and joins again the axial portion of the embryo. I have repeatedly found such conditions. Let us now consider a case in which the cardiac tube ends blindly in a unilateral pericardial cavity. Experiment Type II, No. 14 illustrates such a condition (figs. 3, 5, 9, 10, 39, 41, 42, and 43). The results obtained by this experiment can perhaps be explained by imagining the follow-

3-10 Explanation of abbreviations. *Coel.*, coelom; *Ect.*, ectoderm; *Endc.*, endocardium; *Ent.*, entoderm; *Myc.*, myocardium; *Par. mes.*, parietal mesoderm; *Pcd.*, pericardium; *Ph.*, pharynx; *Pv.m.*, prevascular mesoderm; *Vis. mes.*, visceral mesoderm; *x*, point of fusion of ectoderm and entoderm.

ing process to have taken place: in the anterior potential heart-region the entoderm was probably in close contact with the pharyngeal entoderm as illustrated in figure 7. Such a condition may have obtained as is illustrated in figure 7, which is a hypothetical dorsal view diagram of an early stage in the development of this meroplast. In figure 10 the splanchnic mesoderm is closely applied anteriorly to the wall of the pharynx. Anteriorly no pre-endothelial tissue is shown, just as in figure 7. In the posterior heart-region of figure 7 the splanchnic mesoderm lies farther away from the pharyngeal wall, leaving a space into which pre-endothelial tissue was proliferated. Such a condition must have existed in the posterior region of the cardiac coelom, otherwise the conditions in later development could not be explained. As a possible transition between the stage just described and the conditions actually observed in figures 3, 6, 10, and 39 to 44, figure 8 is submitted. The plug of vascular tissue has increased in length and has enlarged somewhat anteriorly. The visceral mesoderm is giving off a mesially directed fold dorsal to the pre-vascular proliferation, and a similar fold ventral to it. At the same time the visceral mesoderm is invaginating to receive the proliferation. Whatever may have been the actual process involved, figure 9 shows diagrammatically the condition found when the tissue was sectioned; the plane of each section is roughly indicated by arrows in this figure. The blindly ending cardiac endothelium is enclosed by a blind sac of myocardium which projects anteriorly into the coelom. The conditions in the diagram are greatly simplified, and no attempt was made to show the conditions accurately. In this, as in many other instances, there was a number of ridges on the inner myocardial surface in addition to the two principal folds which encircled the endocardial tissue. These ridges are characteristic of cases in which the formation of the vascular tissue is somewhat irregular or considerably arrested. Detailed descriptions of the actual sections are given in another connection.

To summarize the facts just considered, it seems that cardiac endothelium can develop when visceral mesoderm does not

press too closely to the entoderm. When the endothelium starts to develop, the visceral mesoderm gives off mesially directed folds, and perhaps at the same time invaginates in such a manner as to conform to the contour of the endothelial tubes and to engulf the latter by the mesial fusion of these folds. Such funnels, in the heart region at least, are probably not concerned with the production of endothelium, but rather in furnishing it with a myocardial covering. The covering may be found around a single tubular unilateral anlage; but in case the anlage be branched, or a formation from venous rootlets, the branches or rootlets may be provided individually with a myocardial covering. Such formations are probably in process in figures 28 and 29. Here it will be seen that each endothelial tube is partly surrounded by myocardium. These facts are of interest in connection with the phenomenon of multiple heart-formation which is sometimes observed.

For a number of years, cases of duplex heart-formation have been known, and have been ascribed to various causes. Panum explained double heart-formation on the assumption that a pull on the bifurcated ventral aorta had torn asunder an already formed heart. Rabl and others have attributed it to a failure of the bilateral heart-anlagen to fuse into a single heart. Various cases of multiple hearts have been observed. A most unusual case is that described by Verocay in the proceedings of the Deutsche Pathologische Gesellschaft, 1905. He described a case of a chicken with seven hearts (Heptacardia). At a hotel in Cortina d'Ampezzo, an apparently normal fowl was found on evisceration to contain a peculiar clump of tissue which when further examined was found to contain seven hearts. Unfortunately the specimen was badly mutilated. Later examination of this clump of tissue by Verocay (73) revealed the fact that five of the seven hearts were of normal shape and size. The two which were removed from the rest exhibited normal relationships of heart-cavities, valves, and stems of the arterial vessels. It was impossible to determine the relationships of the great veins and the auricles. Verocay believed that each heart had been provided with a separate pericardium of its own. It

is difficult to imagine a process by which such a condition could have been attained. Verocay explained the existence of multiple hearts on the following basis as Rabl and others had previously done: there may have been a failure of the lateral anlagen to fuse; also since each of the lateral heart anlagen normally is a single vitelline vein, it is conceivable that individual hearts had been formed from the rootlets of vitelline veins or from multiple vitelline veins. Gräper points out the fact that in the preparation which Verocay studied there were present several compact knots of liver tissue "which did not represent a mutilated liver, but seemed to be a case of *multiplicatis hepatis* together with *multiplicatis cardis*." Such a condition could be explained on the assumption that the venous rootlets or multiple veins had individually been surrounded and invaded by hepatic proliferation from the wall of the gut. Conditions such as these serve to emphasize the importance of the influence of developing parts on each other—a class of phenomena too often overlooked and greatly underestimated.

Gräper attempted to prove experimentally that duplicate hearts result from a failure of the bilateral anlagen to fuse. This he did by a median incision prior to heart-formation. None of the conditions figured by Gräper give a clue to the formation of more than one heart-anlage on each side. My figures 28 and 29 may perhaps be of interest in this connection. None of my experiments has approached the possibility of producing a seven-hearted condition.

f. The dorsal aorta. Meroplasts produced by longitudinal incisions extending well into the posterior region yielded more or less perfectly developed dorsal aortae which could be traced, sometimes continuously, sometimes discontinuously, to the undoubted dorsal aortae of the head-region. Such findings are of importance owing to the fact that it has been regarded as proved by injection (Evans) that the dorsal aortae of the chick are formed by longitudinal fusion of the elements of the mesial border of the vitelline plexus. Figures 30, 31, 32, 33 and 34 show dorsal aortae or aortic anlagen which were certainly not formed from a vitelline plexus, but are local formations. Such

aortae are sometimes abnormally small, and sometimes abnormally large. That they are lined with true endothelium can hardly be doubted. Their position is sufficiently constant to assure us of their significance.

g. Endothelium independent of mesothelium. The work of Bremer (4) has revived our interest in the coelom theory of the origin of endothelium. Bremer found that coelomic mesothelium furnishes a source of endothelial tissue in the body-stalk of the early human embryo; he found that the body-stalk vascularized before yolk-sac vessels appear. Here was clearly a case in which the blood-vessels did not represent an ingrowth from the yolk. Bremer made the suggestion that all endothelium might arise from mesothelium. He accounted for the presence of sauropsidan vitelline vessels prior to coelom formation on the yolk by postulating their origin from a "premesothelial stage of mesoderm." I have previously pointed out (52) that Rückert has shown in case of isolated blood-islands, that cell groups proliferated from this early vascular tissue cleave to form slit-like cavities which later unite with other similarly formed cavities to contribute to the formation of the coelom.

In my previous work (52) I attempted to produce self-vascularizing meroplasts from regions devoid of coelomic tissue, but was unable to do so. Since that time it has been found possible to operate sufficiently close to the median line in the posterior region to exclude coelomic mesothelium as such, and yet obtain endothelial cavities of local origin undoubtedly representing aortic endothelium. Such a condition is shown in figure 31. In the experiment which yielded this condition, the incision *E-F* (fig. 1) was made in the posterior region of the primitive streak. The longitudinal incision was just lateral to the mesoblastic somite where the mesoderm was unleft. Certainly the aorta here could not have come from an epithelial sort of mesoderm; mesothelial origin seems to be excluded. It might be argued that the somite contains cells that are potentially mesothelium, in analogy with the conditions in lower vertebrates. This may very well be admitted. In fact it is possible, in an earlier stage at least, that any of the mesenchyme cells were

potentially endothelial, mesothelial, dermal, or connective tissue. The formation of endothelium on the teleost yolk-sac by wandering mesenchyme cells a great distance from mesothelium (77, 50, 66, 53) does not speak for a mesothelial origin of endothelium.

2. *The origin of the prevascular mesoderm (mesenchyme)*

Thus far, the present account has not been concerned with the origin of the prevascular mesoderm, except with evidence against the view that this mesoderm is an ingrowth from the yolk-sac vessels. It has been noted that Hahn and Gräper each believed himself to have ascertained which of the primitive layers is responsible for this vascular tissue. It has generally been considered by individual authors that one germ-layer, and one alone, is capable of furnishing this tissue. Even those who believe the direct precursor of endothelium to be mesenchyme would probably derive that mesenchyme from one of the germ layers and from no other. Considerations of the supposedly high specificity of endothelium have given rise to this view; a sponsor of such a view would probably maintain that endothelium is such a highly specific substance that its origin from more than one germ-layer is unthinkable. On the other hand it would probably be maintained that mesenchyme is such a heterogeneous tissue that any degree of equipotentiality in mesenchyme of different sources would be equally unthinkable. Considerations such as these probably explain the fact that different germ-layers as producers of the vascular tissue have generally been regarded as mutually exclusive. Yet when one takes into account the ability with which each of these views has been defended it seems possible that each may contain an element of truth. The immediate section of the present discussion is devoted to a possible reconciliation of the two views—namely the mesodermal and the entodermal origin of the prevascular tissue.

a. *Endothelium from visceral mesoderm*, Let us first consider the posterior axial region. An experimental study as above out-

lined, if concentrated on the posterior axial region, would probably lead to the conclusion (as did the work of Hahn) that mesoderm is the one source of the vascular tissue. Hahn figured a number of cases in which the splanchnic mesoderm was giving rise to ventrally directed cells or cell-groups in different stages up to their transformation into endothelium. Such cases were more numerous and striking in the posterior region; he believed that the same process was involved in the formation of endocardium. Certainly in the posterior region there can be little doubt that such mesodermal proliferation takes place. Here the dorsal surface of the entodermal layer is generally perfectly smooth, seldom having its basement membrane interrupted. In the posterior axial region the incipient endothelial tubes or plugs are in practically all cases from their first appearance more closely associated with the mesoderm than with the entoderm.

It has already been noted that a dorsal aorta formed locally in meroplast Type II, No. 49. Figure 31 shows a section of the posterior axis of this embryo. On the left side the plane of section passes through one of the independent anlagen of the aorta. Anteriorly and posteriorly in the aortic line it is found in numerous places that the mesoderm is proliferating ventrally directed groups of cells, some of which are solid (figs. 32 and 33). The plane of section of figure 32 is much anterior to that of figure 31; it is a higher magnification showing the tissue lying left of the notochord, including the aortic line on that side. The plug of tissue projecting ventrally from the mesoderm is not in continuity with other similar proliferations. Figure 33 shows conditions somewhat comparable to these. This one experiment has yielded all gradations from these solid tissue-plugs to tubular endothelium. Figure 37 shows a series of such down-growths from the splanchnic mesoderm. The plane of section of figure 38 is far anterior to that of figure 37 and the magnification of the latter is greater. From the splanchnic mesoderm in figure 37 one sees prevascular proliferations in all stages up to the formation of what might formerly have been regarded as angioblastic cords, angiocysts or 'sprouts' from these. On the left side of figure 29 there runs mesially from the small cyst a solid

cord of cells which was observed in section to connect with the dorsal aorta; the latter is not shown in this figure. The vascular structures in this figure are not connected with those in figure 38; the two are very far apart in the series. The figures described will doubtless suffice to show the principle involved.

b. Endothelium from parietal mesoderm. It is possible also by experiment to obtain endothelium in a location where it could not have developed from entoderm. An example of such a location is the dorsal surface of the parietal mesoderm. These observations confirm in every way the previous observations of Schulte (63). Here, singly or in groups, cells become dorsally proliferated; before or after losing connection with the mesoderm (figs. 37 and 38) they may acquire cavities, thus forming cysts in every way comparable to those of the splanchnopleure; these cysts may be connected by solid cords of cells or by cytoplasmic filaments. Here we have pre-endothelial tissue completely separated from entoderm by two layers of coelomic mesoderm and by coelomic cavity. There is no observable mesial communication between visceral and parietal vessels, and certainly none by way of the yolk-sac. Obviously we are justified in regarding mesoderm as a possible source of endothelium.

It will be noted in figure 37 that the ectoderm and entoderm have failed to fuse. In instances similar to this I have found that a tube of splanchnic endothelium may grow out laterally until it projects beyond the line of longitudinal incision. This is not only an example of the ability of endothelium to grow, but it is also an illustration of the fact that the direction of growth is not necessarily mesial. In rare cases a vascular tube of the splanchnic variety may send a growth laterally and upwards around the fused mesodermal layers and form a parietal vascular cavity. Actual parietal cavities most often exist, however, which could not have been formed by such a growth, having no connection with the splanchnic vessels, the latter likewise having no connection with yolk-sac vessels.

c. Cardiac endothelium from entoderm? It is a familiar fact that in many forms the endothelium of the heart appears from the first to be closely applied to the pharyngeal wall, often seem-

ing in the normal individual to have arisen from entoderm. A most striking case of this sort was reported by Lee (27). It is of importance to know whether intimacy of endothelium and entoderm is of primary or of secondary significance. It is highly probable that the association of the two tissues is in some way causally connected with the fusion of the bilateral endocardial tubes. This might be brought about in one of, or a combination of two ways. First, endothelium of mesodermal origin might approach and become applied to the entoderm at a very early stage in its genesis; second, this intimacy of connection might be a genetic one; it might be that the assumption of the rôle of vascular proliferation is merely a local adaptation for the process of heart-formation in the amniotes. Either of these two processes might better insure the meeting of the endothelial tubes, following the withdrawal of the intervening entoderm. The immediate section of the discussion is devoted to the second possibility.

I shall now present what I believe to be actual instances of entodermal proliferation of prevascular mesoderm. It may be that I have attached entirely too much importance to these apparent cases of endothelial proliferation by entoderm. If this be true, it has been for the purpose of putting to as severe a test as possible the view in favor of which I have at times been prejudiced—namely the view which regards the mesoderm as the only possible source of the prevascular tissue.

In the posterior region where endothelium is unquestionably of mesodermal origin, it was seen that cells were moved bodily from the more or less ragged surface of the mesoderm. When such cells could first be recognized as forerunners of the vascular tissue they were invariably more closely associated with mesoderm than with entoderm. In the middle heart-region this was often observed not to be the case; the vascular cells seemed from the first to be more closely related to the entoderm than to the mesoderm. At the anterior and posterior limits of the heart, transitions between these extreme conditions exist. Now it is not characteristic of entodermal cells to leave their epithelium and wander about as such in the interstitial regions. If cardiac

endothelium can come from entoderm, one should be able to find entoderm in the act of proliferating that tissue. Such proliferation might well be expected to exhibit mitotic figures. From these considerations it seemed desirable to stain sections of a number of the meroplasts with iron haematoxylin. Mitotic figures in the tissues were generally found to be very scarce; in one case they were found to be almost incredibly abundant (figs. 40 to 44). In this connection it is interesting to note that in a demonstration of material at the thirty-second session of the American Association of Anatomists, Sabin (61) showed that mitosis in chick blastoderms proceeded by definite rhythms, the actual time of mitosis being of exceedingly short duration. In some of Sabin's preparations, almost every endothelial cell could be seen in almost precisely the same stage of mitosis.

As we have previously noted, Hahn stated that the basement-membrane of the entoderm is never interrupted by a cellular proliferation; thus he considered its proliferation of endothelial tissue to be out of the question. Let us now consider certain cases in which the basement-membrane undoubtedly is interrupted. Instances of this are most often found in the heart-region, such as is shown on the right side of figure 39. Internal to the line of longitudinal incision, the entoderm is seen, even at this low magnification, to be greatly thickened. This figure will serve to locate approximately the same parts more highly magnified in figure 40. Here it will be seen that certain entoderm cells are in active mitosis. The basement-membrane of the entoderm is undoubtedly interrupted in two places. The splanchnic mesoderm is thrown into a number of folds, none of the apices of which is in contact with the tissue which the entoderm seems to be proliferating. Between mesoderm and entoderm are lightly staining strands of a coagulated plasma-like fluid, stained only by the counterstain.

Figure 41 is a section slightly anterior to the plane of figure 40. Slightly separated from the entoderm is a bridge of tissue which can be traced back to the entodermal proliferation of figure 32. Traced still farther anteriorly (fig. 42) this bridge of tissue is still seen to be in continuity with the entoderm. Near

the extremity of this projection from the entoderm there is a vacuole in the tissue. To the right of this vacuole the tissue is solid; from this region there can be traced anteriorly through many sections a longitudinally coursing column of cells. Anteriorly this column contains intermittent vacuoles. The splanchnic mesoderm concreseces around this column of cells and (figs. 3 and 43) the apices of the folds produced fuse mesial to the column of cells which has here (fig. 3) taken on the undoubted characteristics of early endothelium. In figure 6 the endothelium is considerably flattened, the myocardial tube has severed connection with the splanchnic mesoderm and now lies inside the pericardial cavity, appearing free in section. Anterior to this (figs. 9 and 10) both endocardium and myocardium end abruptly. The gross structure of this formation has already been considered.

Summarizing the conditions just described, we have here a column of cells, the base of which rests on the entoderm and is being actively contributed to by that epithelium. Whether the entire column has increased by the activity of this one growing-point can not be stated with certainty. It has probably increased also by its own growth. Anteriorly this column of cells possesses the characteristics of endothelium and is surrounded by a myocardium; both end abruptly. Whether the portion which is truly endothelial has come from entoderm, the reader may judge for himself. I strongly favor the interpretation that we have here a true entodermal origin of prevascular tissue.

Of the numerous cases observed in which pre-endothelial mesoderm appeared to be given off by entoderm, I shall describe one more. This is the case of meroplast Type II, No. 148. Figure 44 will serve to locate the region more highly magnified in figures 45, 46, 47 and 48. As we have already seen the right incision was very close to the embryonic axis, leaving little mesodermal tissue on that side. On the left side the incision was farther laterad, so that the tissue which remained could undergo concreescence. The plane of this section is through the potential heart-region. In close contact with the entoderm is an endothelial tube with a dorsally projecting solid process. Let us now

consider sections of this same general region more highly magnified. Figure 45 is a section considerably anterior to the plane of figure 44. Dorsally there is a projection from the entoderm; it is continuous with certain other structures in this region whose pre-endothelial nature can scarcely be doubted. While mitotic figures are by no means wanting, they are not so numerous as those in the case just considered. Figure 46 is a section through the middle heart-region slightly anterior to the plane of figure 44. In fact the endothelial cavity of figure 46 can be traced discontinuously back to the endothelial cavity of figure 44. It will be noted that the endothelial cavity in figure 46 is joined to the entoderm by a solid cord of cells at the base of which an entodermal cell is dividing. In the posterior heart-region (fig. 47), interesting conditions obtain. Dorsally there is a plug of cells, evidently being given off from the entoderm. There can be no doubt that the basement-membrane is here interrupted. More ventrally there is a similar proliferation. The latter is in contact with, but not continuous with the visceral mesoderm. Still more posteriorly the conditions are of great interest. Here there appears to be a transition between a region where the entoderm seems to be the source of endothelium, and the more posterior region where the mesoderm seems to be responsible for all the endothelium. In figure 48 it will be noted that the basement-membrane of the pharyngeal entoderm is interrupted and that pre-endothelial cells lie in a matrix common with that of the entoderm and also with that of the splanchnic mesoderm. Destroy the entodermal connection with this pre-vascular complex, and one would say that this complex had originated from splanchnic mesoderm. Posterior to this region the basement membrane of the entoderm is intact, and endothelial tissue has no connection with any tissue other than visceral mesoderm.

Figure 35 is a section just anterior to the heart-region of mero-plast Type I, No. 34. Such a condition as this would probably be interpreted as an instance of mesodermal origin of endothelium by delamination. This section is more anterior than the region represented by figures 45, 46, 47 and 48; it is the ventral extremity of a left fold comparable to that of figure 44.

Whatever may be the significance of these conditions above described, they demonstrate the fact that projections from the entoderm resembling pre-vascular tissue undoubtedly can be found. In view of their demonstration we have these alternatives of interpretation: either the entoderm is capable of giving rise to vascular tissue, or such proliferations and projections from any tissue whatsoever are to be distrusted as indications of the actual origin of the vascular tissue. If one searches with sufficient diligence, he is likely to become convinced that neither the view of the entodermal origin, nor the view of the mesodermal origin of the vascular tissue embodies the whole truth; conversely, he is likely to be convinced that each view contains an element of truth. The two views are best harmonized by considering the pre-vascular tissue as mesenchyme, whatever may be its source. If cardiac endothelium actually arises from entoderm, the phenomenon may well be considered a mere local adaptation to the circumstance that it is advantageous to have the bilateral endocardial tubes closely approximated in anticipation of their future fusion. The whole question may well remain open, whether one or both layers are capable of giving rise to vascular tissue. It seems highly probably that both layers share in the production of endothelium. Subsequent observation alone can determine the significance which is to be attached to these undoubted conditions which I have actually observed, and have figured as faithfully as possible.

3. The origin of endothelium in hybrid teleosts

Loeb (28) first demonstrated that chemical treatment or hybridization in the teleost would inhibit the development of a blood-circulation, even though the embryo was able to develop a beating heart. Stockard (64) has shown and also Werber (78) has shown in case of chemical treatment, that the lack of circulation is caused by the failure of the independent vascular anlagen to become continuous. McClure (37) has extended these observations to the lymphatics. The effect of hybridization was again observed by Newman (45). Thorington and the writer

(55) have shown that in hybrid embryos of the teleosts the vitality of the embryo is often so low that it is never able to develop endothelium beyond the stage of the independent anlagen. The embryo from which our figures 13 to 15 (pp. 94-96) were taken is of unusual interest, both because of the condition of its endothelium and the position of its blood-cells. Later, Newman² states:

Among the most interesting anomalous conditions seen in these hybrids are the various disturbances in relation to the parts of the vitelline and systemic circulation. The heart and its main vessels frequently appear disjoined from the body and exhibit an independence in differentiation and an automaticity truly striking. Many problems might be cleared up by a study of these conditions.

Incidentally it might be mentioned that my own results obtained from chemical treatment confirm the observations of other observers concerning the origin of endothelium under these conditions.

5. Observations on living teleost material

In the yolk-sac of the chemically treated teleost embryo from which figures 61 and 62 were made, mesenchyme cells were followed in their active migration during their process of transformation into endothelium. Individual cells in some cases aligned themselves into solid columns which later became hollow. Among the more rounded endothelial anlagen a case was observed in which a rather confused group of young blood-cells and intermingled mesenchyme-cells arranged themselves into a vesicle containing blood-cells. Anastomoses between already formed vascular cavities were seen in some cases to be a result of an actual sprouting of the endothelium itself.

In the cardiectomized embryo from which figure 82 was made, the heart was removed while it was a very slender tube. It had never pulsated. The living mesenchyme cells were later followed on the yolk of this embryo. They were migrating from the posterior body-region to the posterior portion of the yolk-sac.

² Newman, H. H., Proc. Am. Soc. Zool., 1915.

All the mesenchyme cells on the yolk which later formed endothelium and blood-cells and chromatophores migrated ventrally and forward beyond a zone comprising the posterior yolk-surface. Thus it is seen that the posterior surface of the yolk is free of endothelium, blood-cells, and of chromatophores. Sections of this embryo show that no endothelial tubes exist in this posterior yolk-region. There is no connection of yolk-sac vessels with those of the embryo's body posteriorly, anteriorly or laterally. The yolk-sac vessels represent a large number of discontinuous spaces with intimately associated chromatophores. In the tail region of living embryos it was possible to follow free mesenchyme cells in the formation of endothelium, though the process was here complicated by the presence of large numbers of mesenchyme cells. These results on teleost endothelium confirm those of Wenckebach, Raffeale, and Stockard.

If allowed to estimate the relative values of mechanical experimentation on chick embryos and chemical treatment or hybridization of teleost embryos, I should say that the former is the more conclusive. In the body of the teleost, it is impossible to say whether discontinuous spaces have always been out of continuity. It is true that there has been observation (66, p. 586) indicating that a vessel once having formed, has little or no tendency to collapse. I was formerly led to believe this to be true. Studies during a subsequent spawning season of *Fundulus* have convinced me that this question of collapsing vessels will bear further investigation. In regard to the yolk-sac vessels of the teleost, it may be stated that no one has doubted the local formation of yolk-sac vessels in any group of vertebrates.

PART II. THE ORIGIN OF BLOOD CELLS IN TELEOST EMBRYOS

As many writers have observed, the actual genesis of the blood is obscured by the fact that blood cells are swept from their places of origin before their differentiation is completed. By the motion of body fluids, blood-cells become swept into places where they did not originate. As Stockard was perhaps first to

suggest, it is evident that if circulation could be prevented without altering the normal process of blood-formation, a great deal of light would be thrown on that normal process. Stockard (55) employed alcoholic treatment as a means of preventing the circulation of body fluids. He found from such study (64, p. 125) that there are "two distinct and limited places of origin" of red blood cells—the stem-vein, and the posterior surface of the yolk-sac. In his writings he repeatedly states in entire paragraphs of italics that if there has been no blood-circulation, the following regions and parts never contain a single erythrocyte in any embryo at any age. These regions include: (1) all the anterior blood-vessels, (2) all the anterior mesenchyme, (3) the anterior yolk-surface, (4) the heart, and (5) the liver. He claims that leucocytes form only in the anterior mesenchyme of non-circulating embryos. Later in the discussion I shall consider Stockard's work in connection with my own; this statement of his results will suffice to show the differences in our findings.

1. Hybrid material

Thorington and I (55) have shown that hybrid teleosts devoid of circulation may develop erythrocytes in practically any region of the body and of the yolk-sac. The embryo from which our figures 13 and 15 were taken is of great interest. The heart was solid at both extremities, yet it contained erythrocytes. Perhaps a blood-lacuna formed as a portion of the heart. The cardiac tube was widely separated from ventral aorta by mesenchyme. The aorta was barren of blood-cells, yet the precardinals contained many erythrocytes, as did the ducts of Cuvier. No blood-cell ever entered the aorta from the cardiac tube. The aorta itself was discontinuous. For further details see the original communication (55).

2. Chemically treated embryos

The following section is devoted to a description of conditions obtained by early chemical treatment of the developing ova of *Fundulus heteroclitus*. The descriptions are based principally

on embryos treated with weak solutions of acetone, butyric acid or potassium cyanide. Generally the ova were placed in these solutions for twenty-four hours or less, and then reared in running sea-water. The results obtained demonstrate that erythrocytes form in the intermediate cell mass and on the posterior yolk; in addition to this, they indicate that erythrocytes may form in any of those locations in which Stockard claims they never form in embryos without circulation. Some of the conditions about to be described have been previously reported (53).

It is a striking fact that under chemical treatment the embryos tend to exhibit abnormalities. Still more striking is the fact that all the tissues in the anterior end seem to be the most seriously affected. It is rare indeed that the circulation can be inhibited without injury to the anterior end of the body. The condition of oedema in the pericardium produced by chemical treatment swells the pericardium, thus stretching the heart until it becomes solid, or until it is unable to propel the blood. A treatment sufficiently rigorous to render the pericardial cavity oedematous is generally sufficient to create marked disturbance in the anterior tissues. By studying a very large number of embryos, however, one is able to find a few cases in which the circulation has been inhibited and yet at the same time the anterior region bears some resemblance to the normal condition. If we concentrate our study on such embryos as these, we arrive at very different results from those of the observer who concentrates on the majority of embryos whose anterior ends have suffered specifically from the abnormal treatment. Whether the anterior end is injured or remains normal, the posterior end of the chemically treated embryo generally is very little deranged. A lethal treatment is often without serious effect on the posterior region. The explanation of this phenomenon is very difficult. It is merely one of the axioms of development which we must recognize. Child (6) deals with this phenomenon under the term 'axial gradient.'

The vascular tissues on the teleost yolk-sac are formed largely from mesenchyme cells which migrate there from the posterior

axial region of the embryonic body. Now it generally happens that conditions which will produce oedematous pericardia will also produce embryos with yolk-sacs whose anterior portions lack endothelium and blood-cells. This fact probably finds its explanation in the circumstance that conditions which will prevent circulation will also vitiate these migrating mesenchyme cells to the extent that they are unable to reach the anterior yolk, or are rendered unable to form these vascular tissues even though they reach that location. But here again it is possible to find many exceptional conditions. Many instances can be found in which migrating mesenchyme cells can reach the anterior yolk and there form blood-cells, even though the heart has never produced any circulation whatever. Thus it is that in chemical treatment, especially if the chemical treatment be severe, a large number of embryos are able to develop red blood cells in only those positions where Stockard claims they can develop. There is most often a coincidence between the absence of erythrocytes in the anterior region of the yolk and the anterior axial region. There are, however, many exceptions in which this barrenness of erythrocytes holds good for only one of these regions, even though the blood has not circulated. If an observer, in selecting his material, uses as his sole criterion for the failure of circulation the absence of erythrocytes in these regions, his method is capable of giving only one result.

Examination of the rather small percentage of embryos devoid of circulation which have been able to develop relatively normal eyes and forebrains often reveals in such embryos a tendency to exhibit locally formed and sometimes bilaterally symmetrical patches of erythrocytes at an early stage in the ontogeny. These patches may be in any position with reference to the eyes, and sometimes even in the optic cups. The time during which they exhibit their red color is usually very short. Sometimes following the appearance of a patch of erythrocytes on one side of the head a similar one would appear on the other side symmetrical with the one first observed, the latter having in the meantime lost its color. During the study of such conditions, all possible efforts were made to detect any movement of red blood cells,

either active or passive. Such changes would take place in embryos whose hearts were solid. The region mesial to the optic cups, and sometimes the snout, displayed such patches of erythrocytes. Blood sometimes developed in the dilated portions of hearts which were solid at both ends. In some cases the heart had solid extremities, but contained a number of blood-bearing dilations.

Figures 49 to 60 are sketches of the dorsal cephalic surface of living embryos whose blood-circulation was prevented by chemical means. In some of these embryos the heart was solid at one extremity or both, or solid throughout. The stippled areas represent these patches of erythrocytes. Whenever such an area was sectioned and stained, the same group of cells could be located in section, the erythrocytes staining intensely red in my modification of Mann's methyl blue-eosin stain. The surrounding mesenchyme would stain a light or intense blue. In some cases the hematopoietic areas found in section were larger than one should expect from the sketches of the living condition. This probably means that cells may stain intensely red even though they are not visible in the living condition. In no case was the eosinophilous region smaller than the group of erythrocytes seen in the living conditions. The method of chemical treatment is either given in the text with the discussion of each embryo or with the explanation of the figures. Figures 49 to 56 show dorsal views of the cephalic regions; figures 57 and 58 are lateral views. The nature of the heart and the distension of the pericardium can be observed in these figures.

In the embryo from which figure 49 was taken, several blood lacunae could be seen on the dorsal surface. Two such layers are located dorso-mesial and two are dorso-posterior to the eyes; a fifth median group is found dorsally located in about the transverse plane of the posterior limits of the eyes. Each of these groups of cells, together with certain others which were invisible in the living condition, were found when the embryo was sectioned. They are illustrated in figures 67 to 70. The heart of this embryo was, from the first, solid at both ends.

In figure 50 are shown two lacunae dorsal and mesial to the optic cups. Their red color could no longer be distinguished four hours after this figure was made. Figures 51 and 52 are sketches of the dorsal cephalic surface of an embryo the history of which is given in greater detail in a previous publication (53, p. 103). At its four-cell stage the ovum was placed in a solution of 50 cc. sea water containing 10 cc. of $\frac{1}{12}$ molecular butyric acid, where it remained for twenty-four hours. It was then placed in running sea-water. On the ninth day a blood lacuna was observed over the left eye (fig. 51). Sixteen hours later this lacuna could not be seen, but another located symmetrically with its former position was observed on the right side. When the embryo was sectioned, eosinophilous areas corresponding to both these lacunae were found in section. The explanation of these circumstances is rather difficult. It seems unlikely that the hemoglobin should leave a cell unless it should disintegrate. It may be that the overlying mesenchyme rendered the first lacuna invisible. Figures 53 to 56 need not be further discussed. They merely show the position of others of the lacunae which were observed in this anterior region. While the location of the smaller groups, and especially that of the single isolated erythrocytes is rather indefinite, there are certain regions rather characteristic for erythrocyte-formation.

Figure 56 shows a very exceptional condition. No case similar to this was found in the entire series of experiments. In this instance, the posterior portion of the embryo was the part affected; as a result it is diminutive in size. The anterior end is relatively normal. Correlated with these conditions we have discontinuous lines of erythrocytes extending into the ophthalmic region, which were not carried there by heart-pulsation. The rudimentary posterior end contains no erythrocytes.

Figure 58 shows a side view of an embryo which, at the four-cell stage, was treated with a mixture of equal parts of 4 per cent alcohol (sea-water solution) and a solution made from 2 cc. of $\frac{1}{100}$ per cent KCN in 50 cc. sea-water. The embryo developed without a circulation. The heart possessed solid extremities. On the twelfth day a longitudinal column of erythrocytes could

be observed through each translucent otocyst. Within the next three days a single median hematopoietic area was observed in the mandible. These conditions continued for some time. Figure 58 shows the conditions on the twenty-fifth day. Blood cells were observed apparently scattered on the floor of the pericardium. Later sectioning of this embryo indicated that the erythrocytes on the floor of the pericardium had formed locally beneath the mesothelium.

It sometimes happens that embryos develop without forming a heart; sometimes there is little, if any embryonic body present. In such cases the entire yolk-surface may be covered with blood cells, diffusely scattered or in groups; such a condition may obtain when there is practically no endothelial tissue present. Such conditions furnish a positive disproval of the claim that blood cells never form on the anterior surface of the yolk if there has been no circulation. Figure 59 shows a condition in which a small portion of the body axis abuts on a coelomic cavity which appears to be a pericardial cavity. No heart was ever formed in this embryo, yet no region of the yolk was free from blood cells. In figure 60 a condition is represented in which no embryonic body ever developed. No particular region of the yolk, even from the earliest stages following chemical treatment, seemed especially entitled to be designated as embryonic body. The blood cells were somewhat scattered, but in some cases they were densely crowded. In the center of the region towards the observer, a blood-mass is surrounded by endothelium.

A very unusual condition was observed in the embryo from which figures 69 and 70 were made. At its four-cell stage this embryo was treated for twenty-four hours with a 3.5 per cent solution of alcohol. On the sixth day a bright red blood-lacuna was observed on the antero-ventral surface of the yolk. No other erythrocytes could be seen on the yolk or in the body axis at this time, though mesenchyme cells in abundance were migrating on the yolk. There was no very extensive system of endothelial tubes. The heart was apparently normal, but its venous end had not connected with any yolk-sac vessel. On the tenth day the blood-lacuna on the anterior yolk, somewhat elongated,

displayed waves of contraction which started in the end nearer the heart and proceeded ventrally to the opposite end. Evidently this lacuna was provided with some sort of myocardium. It had developed a dorsally directed flexure, as seen in figure 69; the yolk endothelium is not shown in this sketch. At this time the churning of the heart could be seen to loosen the closely packed cells in the intermediate cell mass which had begun to acquire a faint orange color. On the fifteenth day a careful examination of the posterior region of this embryo revealed the fact that certain erythrocytes were being dislocated by the movement of the heart, and some could be followed to a large lacuna on the yolk, although there was no complete circulation of the blood. Such cells would oscillate slightly from the motion of body-fluids caused by heart-pulsation. Blood lacunae on the yolk may sometimes be of this sort of formation. The formation of the endothelial tubes and cavities on the yolk was watched in the living condition. As has already been seen, at the age of ten days this activated lacuna, accessory heart, or whatever it may have been, was pulsating feebly. On the nineteenth day (fig. 70) the lacuna or 'accessory heart' was pulsating seventy times per minute, while the real heart, seemingly normal in shape, was pulsating one hundred times per minute; the latter was in no way connected with any yolk-sac vessel. Likewise no endothelial tissue had ever approached, or formed near the accessory heart. The yolk-circulation was established through the normal heart on the twenty-fifth day. At that time the rate of heart-pulsation had greatly increased, especially in the accessory heart. Rather suddenly on the twenty-fifth day the rate of beat in the accessory heart increased to 136 times per minute while that of the normal heart was 126 times per minute. The accessory heart increased its rate somewhat, and then began to decline considerably on the twenty-sixth day, when its rate became slower than that of the normal heart. At this time the embryo was killed. The flexure of the accessory heart had greatly increased. It is difficult to explain the extraordinary manner in which the rate of beat of the accessory heart was changed. It is conceivable that we here have to do with the

failure of an inhibiting nerve-supply to its musculature. Unfortunately I was unable to section this embryo, as it was in some way mislaid. This misfortune was in a measure compensated for by the fact that the observations on this embryo were made under circumstances (The Marine Biological Laboratory, Woods Hole, Massachusetts) in which I could avail myself of the counsel of many investigators, a number of whom were so kind as to examine the embryo in question and verify my observations. At the time at which the sketch for figure 69 was made, the embryo was examined by Profs. E. G. Conklin, H. McE. Knowler, E. V. Cowdry, E. I. Werber and many others. At a time somewhat later after the yolk-sac circulation had been established, the embryo was examined by Prof. C. R. Stockard. This embryo furnishes an example of a completely isolated functional heart containing blood-cells.

Attention is now called to the conditions in one of the most important embryos obtained from the entire study. In this fortunate case the conditions which were able absolutely to prevent the formation of vascular tissues on the anterior yolk, and to cause the venous end of the heart to be solid, did not prevent the formation of erythrocytes in the anterior vessels or in the anterior mesenchyme. This embryo is also to be considered presently in connection with the formation of blood in the aorta and the liver. It seems certain that mesenchyme cells in these various parts were locally transformed into erythrocytes. On the left side of figures 62 and 63 will be noticed a cup-like invagination of the fore-brain tissue resembling closely an optic cup. In the place in which one should expect to find a lens, there developed at an early stage a conspicuously red blood-lacuna. No other endothelium or erythrocytes could be found in connection with this lacuna, either in the living or sectioned material. Dorsal to the optic tissue on the right side of figure 55 are a few erythrocytes which were found lying in the crevices between the peculiar folds of nervous tissue. Some of the cells in these locations seemed to be transforming into erythrocytes, but bore unmistakable traces of a mesenchymal nature. Such isolated cells were found entirely out of relation to all endothelium.

The heart of this embryo (fig. 63) is seen to be solid in its anterior portion. More posteriorly (fig. 62) in the arterial end the endothelium has a lumen. The endothelium again becomes solid immediately at the 'insertion' of the arterial end of the heart on the embryonic body. A section passing through the posterior limits of the diencephalon shows well developed precardinal veins (fig. 64) so closely packed with erythrocytes that the latter have angular contours. Still more posteriorly in the region of the otocysts (fig. 75) the precardinal lines are seen to contain erythrocytes. This section also passes through the very small portion of the ventral aorta which was able to form. Almost immediately posterior to this plane of section, the aorta completely disappears. Besides this small portion of ventral aorta, no portion of the branchial arterial system was found in section. The remainder of the ventral aorta, the aortic arches and cephalic dorsal aortae are entirely absent. Since no vascular tissues developed in the anterior yolk, and no erythrocytes were formed in the heart of this embryo it seems reasonable to assume that these erythrocytes were local formations in the ventral aorta. They may or may not have been contributed to by aortic endothelium.

This embryo received closer observation than most of the embryos studied, for the reason that its peculiar eye-formation rendered it conspicuous even before the blood could possibly have circulated. While a number of mesenchyme cells reached the anterior yolk, no formation of endothelium or erythrocytes took place in this region. This fact was noticed in the living condition and is verified in section. Some of the mesenchyme cells on the anterior yolk would probably have transformed into erythrocytes if the embryo had been allowed to live longer; some of them, still typically stellate, were found to be very slightly eosinophilous. In figure 62 the yolk sac is devoid of vascular tissues. The same is true of all sections anterior to this, and of many which are posterior. Figure 75 shows the most anterior extent of erythrocytes that were found on this yolk-sac. These were far dorso-lateral, and belong to an isolated lacuna.

In this embryo the solid distal end of a very feeble heart ends blindly on a yolk sac the anterior portion of which is, and has always been devoid of vascular tissues. Yet the anterior mesenchyme and the anterior vessels contain blood cells. As will be shown later, the liver of this embryo contains erythrocytes, though these may possibly be mesenchyme cells pushed out with and included in the liver diverticulum.

The conditions observed in figure 49 may now be considered in section (figs. 65 and 66). In each case the position of the blood-producing area is illustrated by solid black dots, while in the illustrations of these regions more highly magnified the erythrocytes are shown by unstippled cells. It is quite impossible to do justice to the preparation by means of one-color illustrations. The erythrocytes stained a brilliant red, the mesenchyme cells a rich blue, while the probable transitional stages between the two stained purple. The erythrocytes seem to be identical in nature with those in the intermediate cell-mass. Figure 65 shows a section taken just posterior to the optic vesicles. The erythrocytes in this figure are not surrounded by endothelium; there is a large group on the left side, and two groups on the right. The latter are shown in greater magnification in figure 67, where instead of the arbitrary black dots we have unstippled cells representing erythrocytes. Some of the cells surrounding this group of erythrocyte groups seem to be flattening out to form endothelium.

In figure 66 we have a section through the dorso-median blood-anlage observed in figure 49. These erythrocytes (fig. 68) lie in a crevice in the fore-brain tissue; they are not surrounded by endothelium, but are bounded entirely by ectoderm. No endothelium developed in any region near this group of cells. It is even doubtful whether one should find endothelial tubes in this region in the normal embryo at a stage corresponding to this.

It is of interest to note that Professor McClure, working with the fresh-water teleost *Erimyzon sucetta oblongus*, has obtained results similar to those above described for *Fundulus*. He obtained chemically treated embryos without circulation, having lacunae of erythrocytes in the anterior mesenchyme. His ex-

periments, in fact, preceded my own, but his results were unknown to me until my return from the Woods Hole laboratories. He has kindly placed at my disposal an embryo on which he made daily observation, and which he sketched in the living condition. In the living embryo a group of erythrocytes was observed dorsal to each eye. A section through that region is shown in figure 76. On the left side the erythrocyte group is the more prominent. It is not surrounded by a distinct endothelium. In sections of this region, such crescent-shaped groups of erythrocytes over the eyes are not of rare occurrence. They are found sufficiently often to indicate that the mesenchyme of this region may be a true erythrocyte-anlage. On consulting Werber's figure 24 (78) it will be seen that he found a blood-anlage very similarly located. He describes the blood cells in this region as leucocytes "evidently of polymorphonuclear variety," subscribing at that time to the view of Stockard that the blood cells produced in the anterior region are leucocytes. I regard it doubtful that these are really leucocytes. Be this as it may, the homologous region of the mesenchyme which in Werber's figure seemed to give rise to leucocytes gave rise to erythrocytes in the experiment performed by Professor McClure. It will be seen in figure 77 that the arterial portion of the heart is solid; sections show this embryo to be devoid of ventral aorta, aortic arches, and anterior dorsal aorta. Examination of sections somewhat posterior to the eyes reveals a region posterior to the erythrocytes on both sides of figure 76, which contains no haemal vascular tissue whatever (fig. 78). Thus we see that the vascular anlagen anterior to this are completely isolated. A section through one of the nasal pits of this embryo (fig. 79) shows a small vascular cavity containing a few erythrocytes. The endothelium of this cavity narrows down to an exceedingly fine tube which can be traced back to the region of the vascular anlage on the same side in figure 77. The tissue in this embryo is in a remarkable state of preservation. It was stained in Delafield's haematoxylin and orange G. The differentiation of the erythrocytes is not very great in this stain, so that they might easily

be overlooked. In photograph they stand out more clearly than to the eye; this was accomplished by the use of color-screens.

Let us now consider certain embryos devoid of circulation which developed erythrocytes in their hearts. In these instances the embryos were watched carefully at short intervals. It is quite unlikely that a heart, partly solid, would hollow out between these intervals only to become solid again at such intervals. If a blood-cell be found in the heart of an embryo in which there had never been a circulation, there are at least the following possibilities of its origin: The fully formed erythrocyte may have reached that location (1) by its own independent power of locomotion, (2) its mesenchymatous anlage might have reached that location by active migration, (3) the erythrocyte in question might be a transformation of an endocardial cell, (4) the erythrocyte in question might be a transformation of a cell that would ordinarily have become a myocardial cell, (5) it might be a transformed mesothelial cell. These seem to include most of the possibilities. It is evident that if an investigator should state that in the absence of circulation the heart "never contains a single erythrocyte in any embryo at any age" he would relinquish all right to claim that an erythrocyte found in that location had reached it by any of the five possibilities just outlined. A claim that a blood cell never develops in a given location excludes the possibility of claiming at the same time that a blood-anlage had migrated into that location.

The conditions in the heart of the embryo from which figure 49 was taken is of interest. No circulation could be observed in this embryo. On the fifth day it was noted that both extremities of the heart were solid, though it appeared otherwise to be normal. Previous to this time the embryo had received frequent examination. The material seemed very favorable, owing to the fact that its condition seemed unusually near the normal, considering the fact that there was no circulation. The pericardial cavity was not greatly oedematous, and the heart was not stretched. In fact it possessed a well-developed flexure. The venous end of the heart was undoubtedly solid, and no yolk-sac endothelium approached it. A means suggested itself of pre-

venting any elusive sort of circulation which might take place between the intervals of time at which any single investigator could reasonably be expected to examine this embryo. If the heart could be severed, such a circulation would be precluded. A very fine needle was used to sever the heart at its middle portion. The cut ends fused laterally, the fusion being intimate in some places and loose in others. In figure 66 the solid distal portion of the venous end of the heart is shown ventral to the head. On the right side of a section of the heart of this same embryo (fig. 65) taken more posteriorly there will be seen a continuation of the venous portion seen in figure 66; this right portion, followed posteriorly, disappears abruptly. The left portion of this same section pursued posteriorly is found to be the arterial end of the heart which enters the embryonic body; but if the left portion of the heart of figure 65 be traced anteriorly it disappears abruptly, as did the venous portion when traced posteriorly. The two portions are everywhere in the region of fusion well separated mesially.

Figure 71 is a section taken slightly posterior to that of figure 65, near the plane in which the venous portion ends abruptly. In the venous side there are seen to be numerous erythrocytes apparently in all stages of differentiation. Some of them have large nuclei and stained intensely red; others with medium-sized nuclei stain purple; still other rounded cells with small nuclei stain blue. It will be noticed that there is no endothelium and also no myocardium in this portion of the heart, whereas erythrocytes are plentiful. On the left side, no erythrocytes are seen in section; a few scattered erythrocytes can, however, be found in this arterial portion nearer to the embryonic body. It will be noticed that the endocardium in this portion of the heart exhibits a cuboidal form very unlike the normal squamous epithelium. Conditions such as these are often found in these chemically treated embryos. Undoubtedly this is an abnormal condition, but it must have some significance.

So far no attempt has been made to give the source of the erythrocytes found in the heart. The conditions in the heart of the embryo from which figure 55 is taken may be of interest as af-

for a possible clue to the origin of such erythrocytes; there may be those who would be willing to consider these conditions as evidence that cardiac endothelium can form erythrocytes. If it had been possible to anticipate the unusual importance of this embryo its history would have been kept more fully. With a number of others which had received like treatment this embryo was segregated to a dish from which all embryos were removed as soon as they were observed to develop a circulation. Later it was removed to a dish in which embryos were placed only on condition that their otherwise normal endocardia should be solid at both extremities. Thus it is certain that this embryo once possessed a complete endocardium which was solid at both ends and dilated in the middle. On the eleventh day the upper end of the heart was seen to display developing blood-cells which at first were orange-colored. This color gradually deepened into red by the fourteenth day, when the embryo was killed. Sections of the heart reveal very interesting conditions. Figure 72 shows a section through the distal (venous) end of the heart not far from the point at which the endothelium is solid. The endothelium is here of a cuboidal type similar to that in figure 71. It stained purple. Inside the endothelium are a number of erythrocytes which stained bright red. Outside the endothelial cells lie the myocardial cells which stained pale blue. The plane of section in figure 73 passes through a flexed portion of the cardiac tube. Some of the erythrocytes in this section lie in a column of erythrocytes which can be traced back to those in figure 72. It will be seen that the endocardium is interrupted. Some of its cells are undoubtedly transforming into erythrocytes. At the right of the lower extremity of the V-shaped remnant of the endocardium on the left of the figure there are abundant transition stages between endothelium and erythrocytes. From the main central mass of erythrocytes in this figure there appears in section a rather sharp 'projection.' In this it is easy to follow the contour of what probably was once the endocardium. The cells at the apex of this projection stained a reddish purple. In the middle of this figure it is seen also that some of the myocardial cells have also undergone this transformation, especially in the

upper part of the figure. In figure 74 which is still more proximal is shown a region in which both endocardium and myocardium have completely transformed into erythrocytes; the entire content of the mesothelial covering consists of strongly eosinophilous cells of a typical erythrocyte character. It is a region in which endothelium has been observed in the living condition in this embryo.

3. Hematopoiesis in the liver

Stockard believes himself to have demonstrated that liver tissue is incapable of forming blood cells, and that it has no hematopoietic function in the bony fishes. His conclusions are based on the fact that he failed to find blood cells in the livers of chemically treated embryos which developed without a circulation. If this conclusion necessarily follows, one might claim that the actual finding of erythrocytes in the liver of an embryo which had not had a circulation would prove the liver to be a blood-producer.

One question which should be settled is this: are embryos devoid of circulation capable of developing livers to a stage in which their potentialities are given a fair test? Even though the chemically treated embryo may retain its life for a time equal to that in which the normal liver appears to produce blood-cells, it is questionable whether the conditions in the chemically treated embryo can ever approach the normal condition.

If a blood cell should develop locally in the liver it might be of mesodermal or of entodermal origin. The vertebrate liver is morphologically a growth into the ventral mesentery; consequently it has a covering of mesothelium and of mesenchyme. The liver of *Fundulus* is a growth of the gut into the mesenchyme which in turn is covered by mesothelium. These mesodermal tissues are properly considered a portion of the liver tissue. If any of the mesodermal elements of the liver should turn into blood cells, such transformations must necessarily be reckoned with in the study of the liver. It is conceivable that hem-

atopoesis in the liver might also consist in a direct transformation of entodermal cells into blood-cells.

There seems to be abundant evidence that mesenchyme cells caught up in the liver tissue are capable, just as is true of other mesenchyme cells, of turning into erythrocytes. This alone does not render the liver a 'hematopoetic organ.' Applied to any structure which embryos devoid of circulation are capable of developing, the term 'hematopoetic organ' is probably a misnomer. Figure 81 shows a section of the liver diverticulum. A part of the tissue in this section lies near the junction of the diverticulum with the gut. The erythrocytes which developed in the periphery of this liver may have come from mesenchyme cells captured by the outpushing entodermal tissue. This figure is from the embryo of which figures 62, 63, 64 and 75 represent sections. Reasons have already been given for the belief that this embryo never developed a circulation. The erythrocytes in figure 81 are neither surrounded by endothelium nor dangerously near any endothelium. It is impossible again to illustrate adequately the differentiations in these tissues by the use of one color. The erythrocytes are brilliant red, the liver tissue is grayish purple, and the mesenchyme is pale blue. Within the liver are certain large cells more coarsely granular than the surrounding cells; their nuclei are larger than those of the surrounding liver-cells, and somewhat eosinophilous. The nuclei of the adjacent liver cells are dense bluish black. The cytoplasm of these larger cells has assumed a purplish pink color. Some of these cells, or at least some of the cells located near them, approach rather closely the erythrocyte characteristics. In some sections of this liver it is impossible to tell whether the erythrocytes present have developed from cells similar to these, or from mesenchymal inclusions.

Figure 80 shows a section through the liver of the embryo of *Erimyzon* from which figures 76 to 79 were also taken. Lying promiscuously in the liver tissue are differentiating erythrocytes which are out of relation to all endothelium. It seems improbable that the partly solid heart of this embryo could have caused

a sufficient negative pressure to have moved these erythrocytes to their present location from another region of the embryo.

Numerous other cases were observed in which here and there a captured mesenchyme cell had probably changed into an erythrocyte. It is likely that the results of chemical treatment tell us little more concerning the potentialities of the fully developed liver than they tell us of the potentialities of spleen and bone-marrow.

4. Ova subjected to low temperatures

Another means of preventing blood-circulation in *Fundulus* is to subject the ovum to low temperatures at a very early stage of development. This method of developmental arrest is not new. Four-celled ova were kept at about 4°C. for periods of time ranging from twenty-four to seventy-two hours. Development under these conditions proceeded rather slowly. Embryos often developed without circulation. In all probability the lowering of the temperature causes a blastolysis (Werber, 79). The yolk-sac in such embryos is often greatly shrunk away from the chorion, and evidence of egg-extrusions can often be found in the subchorionic space. Embryos so treated often fail to develop hearts. It may be that the region later to produce the heart-anlage in such cases becomes blastolyzed. At any rate, these embryos devoid of hearts are of interest owing to the fact that their anterior mesenchyme is sometimes hematopoietic. I have obtained erythrocytes in such embryos in positions similar to those shown in figures 50, 54, and 55.

5. Cardiectomized embryos

Removal of the heart prior to heart-pulsation is the ideal means by which one may assure himself that no elusive circulation will falsify the picture obtained in the study of blood-development. The heart region may be destroyed even before a heart-anlage can be found. If this be done the question of passive movement of the blood-elements can be eliminated. The operated embryos may be placed in any position with reference

to gravity, and it is possible to obtain embryos from which body-movements can not be elicited. The method has other advantages. The only unfavorable chemical effect is produced by the lack of tissue nourishment and tissue respiration, and the accumulation of katabolic materials together with the possibility of infection. Unfortunately these are not always insignificant. The rate of mortality from such operations is very high indeed, probably because of infection. • But once an embryo is able to recover somewhat from the operation it furnishes valuable material for study. In chemical treatment the specific ill-effects of the chemical used are to be added to inanition, the lack of tissue-respiration and the accumulation of katabolic products. Furthermore in case of cardiectomy the embryos may in some instances be allowed to develop normally for seventy hours before operation is necessary to prevent circulation, while in chemical treatment the entire development for the first one to three days must take place in a very abnormal environment. But the one characteristic in common with the results of both sorts of treatment is that the anterior region of the body always suffers the most. It seems practically impossible to obtain cardiectomized embryos whose anterior tissues are perfectly normal, though their posterior regions are generally little injured. In some cases a very small portion of the head was severed from the rest of the body and from the heart; it was hoped that the mesenchyme in such meroplasts would be able to form erythrocytes. It was found, however, that these head-fragments would not increase in size. Thus it was found later that the diameter of such fragments was always much smaller than that of the remainder of the body (figs. 84 and 85). The mesenchyme in these small fragments always became very closely packed, so that it is not surprising that blood-cells were unable to develop under these conditions.

There is reason to believe that in some instances the conditions obtained by cardiectomy may represent more faithfully the normal process than can be obtained by chemical treatment. The former method is capable of producing stages more advanced in differentiation and growth than one can obtain by chemical

treatment which will prevent circulation. If differentiation can proceed further in cardiectomized embryos, it is not unreasonable to believe that the process there is more nearly normal. It is practically certain that the normal process of hematopoiesis is not duplicated in either case of abnormal treatment. So far, the most anterior erythrocytes obtained in cardiectomized embryos are located ventro-mesial to the anterior portions of the otocysts. None has yet been found in the ophthalmic region and in the snout. These experiments were not begun, however, until late in the spawning season of *Fundulus*. Fifteen hundred cardiectomies were performed with only partial success, so that it is impossible to predict at present the possibilities of this method of experiment. A very few embryos survived the operations. Those embryos which fail to withstand such treatment generally die soon after the operation. In most of the survivors, erythrocytes could be seen in the living condition much anterior to the pectoral fins. Most of the present descriptions are based on the living conditions. So far, very few of such embryos have been sectioned. Thus it is conceivable that some of the embryos now preserved may exhibit erythrocytes farther anteriorly than have yet been figured in case of cardiectomy. Only three of the embryos will be described at present.

Figure 82 shows the living condition of a nine-day embryo, the heart of which was removed when the embryo was sixty hours old. The heart had not yet pulsated. Following the operation the embryo was kept in a moist-chamber in a cool place. The tail of the embryo became rather long and curved over the dorsal body-surface. The embryo could not be provoked to reflex-responses, and never exhibited any movement. The pectoral fins were relatively huge, working in unison with a quivering fan-like movement. Very well differentiated striated muscle developed at their bases. It will be noted that the head is relatively small; at the anterior limits of the otocysts it narrows down so that its diameter in the region of the eyes, as seen from the dorsal side, is less than half that of the region of the otocysts. The eyes are merely rudimentary optic stalks. There are neither optic cups nor lenses. The pericardium is oedematous. It con-

tains no trace of a heart except an exceedingly minute vestige of its arterial 'insertion' which can be seen only in section. The erythrocytes visible in the living condition are conventionally represented by means of black dots. A column of erythrocytes formed in the intermediate cell-mass. Endothelial cavities, many of which were surrounded by pigment cells, are represented as dark ramifications on the yolk. There are no continuous channels. Large numbers of erythrocytes developed in the immediate region of the pericardium, and some developed beneath its mesothelial lining; no erythrocytes or endothelium developed on the posterior yolk-surface. Sections of this embryo reveal no connection of systemic and yolk-sac vessels. Ducts of Cuvier, as well as vitelline veins, are absent. The posterior yolk is seen in section to be quite free of vascular tissue. Owing to the translucency of the otocysts it was possible to locate the columnar hematopoietic region mesial to each otocyst. A section through this region (fig. 83) shows the position of these cells ventral to the mesial portion of each otocyst. Traced posteriorly these columns disappear, having absolutely no relation to the intermediate cell-mass. The brain tissue is somewhat abnormal. The pharynx and cartilaginous gill-bars are well developed. The abnormal filamentous processes from the pharynx contain numerous darkly staining spherical bodies which when examined at a higher magnification are found to contain small darkly staining globules. These bodies resemble yolk globules somewhat in their staining reaction. In abnormal cases these bodies often become imbedded in the mesenchyme, and might be wrongly interpreted as leucocytes or lymphocytes. Ventral aorta and aortic arches are entirely absent. Dorsal aorta is encountered only in a region much posterior to this.

Figure 84 shows an embryo in which the heart-anlage was destroyed before it could be distinguished. At the same time a small portion of the head was isolated. At this time, optic vesicles could not be distinguished. An eye developed from the fragment of fore-brain tissue which remained. The otocysts were destroyed. There is no trace of a heart. Erythrocytes developed discontinuously in the intermediate cell-mass. Pro-

jecting diagonally forward and upward in the median plane from the postero-dorsal portion of the yolk is a slender group of erythrocytes, the nature of which has not yet been determined, as the embryo has not yet been sectioned. It is certain that these cells are not a part of the intermediate cell-mass as such. It will be noted that the dorsal portion of the trunk contains pigment. The clear region ventral to this is filled with a clear fluid. The pectoral fins are well developed. The mesenchyme anterior to the pectoral fin is hematopoietic. On the posterior yolk are a few large blood-lacunae. Some smaller ones are found near the diminutive pericardium. The region between these two blood-bearing areas of the yolk does not contain endothelial tubes. The intersomitic grooves are distinguishable in this embryo; such is rarely if ever the case in chemically treated embryos.

Rather unusual conditions were obtained in another cardiectomy (fig. 85). Before a pericardial cavity had been formed, and before heart-formation had taken place a very fine needle was introduced ventral to the head-tissue. Not only was the future heart-tissue destroyed, but the needle was then manipulated so as to sever the body axis in the mid-brain region. The needle did not puncture the body-wall ectoderm. A large spherical oedematous space probably corresponding to the pericardium has formed; instead of forming anterior and ventral to the head-fragment it has formed behind the latter, so that the head fragment has been pushed considerably forward. The diameter of the isolated cephalic tissue has increased very little beyond that at the time of operation, whereas the main part of the body has grown a great deal. The otocysts were partly destroyed, and the remains of them have probably moved forward somewhat. There was no heart-formation. Erythrocytes were observed ventral to the otocysts and anterior to the pectoral fins. An area probably indicating the point of emergence of the ducts of Cuvier from the embryonic body contains erythrocytes. The intermediate cell-mass is hematopoietic. The yolk-sac contains numerous endothelial vesicles and pigment cells, but no erythrocytes were detected on it in the living condition.

6. Transplanted meroplasts

It has been seen from Stockard's work and my own that chemical treatment will often produce embryos, the anterior and ventral portions of whose yolk-sacs are practically free from endothelium. These embryos may at the same time have a slight dorso-lateral circulation through the ducts of Cuvier and the dorso-lateral yolk-capillaries. A number of such embryos were selected. To the endothelium-free area it was possible in a few cases to transplant an anterior fragment of a normal embryo which had not yet had a circulation. The procedure was as follows: the chorion of the 'host' was partly removed over the endothelium-free area. If the rotation of the yolk-sac became troublesome, it was possible to prick the latter in some region where the injury would not be serious. The slight extrusion would hold the embryo in place. A very slight abrasion was sometimes made on the yolk-area where the fragment was to be transplanted. A small receptacle for the tissue was then placed in a wide-mouthed bottle provided with a cork perforated to permit the intake and outlet of gases. Oxygen was then generated and allowed to replace the greater quantity of air contained in the bottle; the latter was then sealed. The tissue was moistened from time to time with sea-water which had been boiled and cooled. The oxygen supply was renewed at such times. On the whole the experiment was quite unsatisfactory. The tissue would generally either die or become invaded by endothelium.

We may consider, however, the most successful attempt which was made. The procedure was similar to that above described. The anterior region, including the otocysts, was transplanted so its ventral surface rested against the exposed yolk-area of the 'host.' The anterior end of the meroplast was directed anteriorly. The transplanted tissue was badly mutilated, although all possible precautions were taken.

Figure 86 shows a section of the transplanted tissue and its position on the yolk. The morphologically dorsal surface of the tissue lies toward the lower left hand corner of the figure. If the figure be rotated until this corner becomes uppermost, one

can orient himself with reference to figure 87, which is the same section of the meroplast somewhat enlarged. The orientation of figure 88 is similar to that of this figure. Examination of figure 87 shows that the plane of section is through an otocyst, probably the left. There is a very thin nervous epithelium partly surrounded by a cartilaginous otic capsule. Laterally located is evidently the anlage of a semi-circular canal. Strangely enough the ganglionic mass accompanying this otocyst lies lateral to it. The tissue on the right side of the otocyst is a rather dense mesenchyme, some of the cells of which are spindle-shaped and massed into rather fibrous complexes. This region to the right of the otocyst represents the median axis of the transplanted tissue. This section contains neither endothelial tissue nor corpuscles. This is true of the remaining posterior portion of the meroplast. The other figures shown are from planes anterior to this.

It will be noted that the yolk-mesoderm 'ventral' to this meroplast has become fibrous. Higher magnification of this tissue (fig. 87) reveals the fact that there are numerous muscle fibers. These seem entirely comparable to those always observed ventral to the anterior portion of the normal embryonic body. It seems that the presence of this anterior axial tissue brings about the formation of muscle fibers. These are, in all probability, *in situ* differentiations of the yolk-sac mesoderm and not migrations from the mesoderm of the transplanted tissue. This point is difficult to prove.

The most anterior section figured may now be considered. The plane of section (fig. 88) is near the anterior extremity of the meroplast. The axial portion is solid cartilage. To the left of this the tissue contains some ganglionic material and mesenchyme, which lies anterior to the otocyst region of figure 87. Within the next few sections anterior, this region on the left entirely disappears, leaving the cartilaginous axis alone in section. The region between the solid cartilage of figure 87 and the axial portion of figure 88 contains mesenchyme cells differentiating into erythrocytes. The cells are rather scattered and none of their groups is large. Figure 89 shows a section taken

between the planes of figures 87 and 88. The mesenchyme stained clear blue while the erythrocytes stained red. Intervening stages (*Erthbl.*) take a purple stain. It seems altogether improbable that these erythrocytes have reached their present location through the circulation. The only blood-vessel which could be seen to approach this meroplast was a spur which projected towards its anterior extremity, but never reached it. Also the hematopoietic area is separated from the vessel by solid cartilage. The posterior region of the meroplast contains no vascular tissue. The transplanted tissue is bounded ventrally by a compact sheet of muscle.

Now this region of the yolk to which the meroplast was transplanted is one of those regions in which the mesoderm, according to Stockard, is incapable of forming blood-cells. It is one of those "regions in which wandering blood anlagen never make themselves manifest." If there was no circulation of the blood, the erythrocytes must have differentiated locally. But this mesenchyme is cephalic, and cephalic mesenchyme, according to Stockard, can not form erythrocytes. The reader may judge for himself which (if either) of these claims of Stockard seems questionable in view of the conditions here described.

The embryo from which the meroplast was removed was preserved at the time of operation and sectioned to make sure that none of the intermediate cell-mass had been transplanted. The posterior end of the meroplast proliferated a great deal of tissue which evidently induced a very active migration of mesenchyme from the posterior region of the 'host.' The region between the two contains a considerable amount of tissue of a nondescript sort.

By no means is an attempt made to estimate the value of this experiment for the solution of the question at hand. The work of transplanation has not been carried far enough to demonstrate its true worth. Greater experience in the manipulation of this method might lead to results which would be truly important. The main trouble lies in the extremely low viability of the isolated tissue.

II. GENERAL CONCLUSIONS

1. Does abnormal treatment obscure the normal process?

At this point I wish to consider certain phases of Stockard's work, taking up first his results, and then the conclusions which he drew from them. In commenting on his previous work (65) he stated (66, p. 583) that this work seemed "in the light of past literature to render highly probable, if not to prove, the polyphyletic origin of the various types of blood-cells," thus disposing of the "now extremely improbable monophyletic view." He regards his technique of study as so highly satisfactory that "the only disadvantage is that the worker may be led to wonder whether so apparently a simple problem is of scientific importance." He believes himself to have disproved the monophyletic view by demonstrating that erythrocytes of the teleost arise only in the intermediate cell-mass and on the posterior yolk; that leucocytes originate only in the anterior end of the body; that erythrocytes can not develop from endothelium, from anterior mesenchyme, in anterior vessels, on the anterior and ventral yolk and in the liver.

The first question which every one must decide for himself is, whether he will accept the introductory statement of Stockard (65, p. 234) that "it can not be argued, so far as the blood anlage is concerned, that the conditions recorded are pathological or other than those which would occur in the normal genesis of the blood except that it never circulates." If it be true that such argument is impossible, then the whole question is settled once for all. There would seem to be a possibility that it can be argued, and perhaps even proved that the blood anlage has shared in the afflictions which are evident in all the other tissues in Stockard's figures. What would exempt the blood anlagen from the ill-effects demonstrable in the other tissues? These are questions which one should answer before proceeding to other considerations.

From Stockard's own experiments and the experiments of many other observers, it is evident that the anterior end of the

embryo is particularly susceptible to the action of chemicals or to any condition which impairs metabolism. Child (6) has shown that here the rate of metabolism is particularly high. It is very common to find the anterior end greatly retarded or highly abnormal while the posterior end of the embryo may be practically normal. Certainly we may say of the nervous, muscular, skeletal, and alimentary tissues that the characteristics which they exhibit under chemical treatment (see many of Stockard's figures) would give a rather imperfect idea of the normal process. The anterior tissues may be so badly upset as to be scarcely recognizable, while the posterior tissues may approach the normal. The investigator who maintains that it can not be argued that the conditions recorded by Stockard are abnormal 'so far as the blood anlage is concerned' must prove that blood is such a stable tissue as to be able to withstand these ill-effects and develop normally, regardless of the conditions which influence so profoundly the other tissues, especially the anterior tissues. A comparison of Stockard's figures 10 and 11 (65) is particularly appropriate at this time.

Stockard's interpretations are based upon the proposition that if under unfavorable conditions erythrocytes can differentiate in one place in the body, all other regions failing to produce erythrocytes under these same conditions are demonstrably powerless to produce erythrocytes under any conditions whatever. Such a view does not permit of different degrees of hematopoietic tendencies for different regions at a given time; it is claimed that any region which would ever produce blood cells at all must necessarily produce them under the given abnormal condition. In other words, if erythrocytes form at all they must necessarily form in all places capable of producing them at any time under normal conditions. From the fact that endothelium grown in an alcoholic environment was able to produce no erythrocytes, Stockard (65, p. 276) maintains that "one is warranted in making the bold assertion that the endothelial lining of the heart and aorta is perfectly incapable of giving rise to any type of blood-cell." As a matter of fact it has never been maintained that endothelium is an important source of erythrocytes as com-

pared with many other blood-producing tissues. The relatively few cases of this sort are generally regarded as significant principally for the evidence which they afford of the non-specificity of endothelium.

It is conceivable that a tissue whose hematopoietic tendencies are weak would be the first to be affected by abnormal treatment. Stockard reasons somewhat differently. He states that "if vascular endothelium had such power, then one might expect that this power would show itself where it is most needed, for example in these embryos in which the blood never circulates." One might take the equally teleological, but opposite view that these embryos are the very ones which do not need erythrocytes. Stockard goes still farther and maintains (65, p. 311) that the alcoholic environment should be able to stimulate endothelium to form blood cells, provided endothelium is capable of being so stimulated. He points out that no endothelial cell has ever been seen to give rise to a blood cell. Should this observation ever be made, it becomes of interest to know whether the phenomenon would be regarded as adequate proof of the ability of endothelium to form blood, or whether it might be regarded as an instance in which the blood anlage was 'exotic' (65, p. 395) or one in which the process of development had not been clean-cut (65, p. 292).

Concerning the following points it is difficult to determine definitely the interpretation to be placed on Stockard's statements. He figures leucocytes in very young stages, yet (65, p. 309) regards their late appearance as significant. He states (65, p. 280) that they have never been observed except in the anterior region of young embryos, yet believes (65, p. 284) that they are of a wandering disposition, later making their way into all parts of the embryo. Rather strangely, on page 269 (65) he states that "in no case has any type of lymphocyte or leucocyte been present in these yolk-islands except as late wandering cells," whereas he repeatedly states that yolk-islands are always devoid of such structures in the absence of circulation. He regards endothelial cells equally specific with blood, and far removed genetically from connective tissue cells and blood cells; he classes endo-

thelial cells with these latter derivatives as being equally specific with pancreatic cells and liver cells (65, p. 323). He assures us that an invisible destiny shapes the ends of pre-endothelial cells, yet on page 316 (65) he states that the mesenchyme "may be admitted to form endothelium largely as a response to physical conditions." He states that erythroblasts never divide when inside an endothelial cavity, and that red blood cells are always formed in or budded into a vascular space; both these statements are probably incorrect.

There are some interesting conditions in Stockard's own material which he seems to have misinterpreted. Let us examine first his basis of belief that no anterior vessel ever contains a red blood cell if the blood never circulates. We must first agree on what is meant by, and included among the anterior vessels. Doubtless the pre-cardinal veins may be regarded as anterior vessels; doubtless also the ducts of Cuvier would serve as a place of demarcation between anterior and posterior vessels. In all of Stockard's figures and discussions the terms 'precordial,' 'duct of Cuvier' and 'post-cardinal' are entirely wanting, so far as I am aware. No term more definite than cardinal vein or stem-vein is used. In the normal individual Stockard misinterpreted the ducts of Cuvier as vitelline veins. He figured vitelline veins arising between the otocysts. If we admit that the adult teleost has a duct of Cuvier, as is generally believed, then the 'vitelline vein' figured by Stockard are ducts of Cuvier whose sources between the otocysts are the precardinal vein. The term 'vitelline vein' is correctly used to distinguish particular yolk-sac vessels of a very definite morphology. Thus in the normal individual, Stockard misinterpreted the ducts of Cuvier, while in most of his experiments its development was prevented, so that in neither case was he able to utilize the only satisfactory landmark by which to determine the anterior vessels. No doubt the embryos in many of Stockard's figures have erythrocytes in their precardinals. Figures from Wenckebach³ and especially those of McClure (36) demonstrate the fact that in young stages the teleostean duct of Cuvier is well posterior to

³ Hertwig's Handbuch, p. 1131.

the otocyst. See also Reagan and Thorington (55), figure 15. The 'splitting' which Stockard often found in the anterior portion of the 'stem vein' probably represents precardinal veins. The 'cardinal vein' of Stockard's figure 9 (65) is almost certainly a duct of Cuvier with its anterior continuation, the precardinal.

At the thirty-second session of the American Association of Anatomists, the writer presented evidence that erythrocytes may develop locally in many regions in which Stockard claims they never form. The objection was immediately raised that the red blood cells observed in these positions had migrated. Such objections had been anticipated (53, p. 116) and answered as follows: "I am not at present concerned with the possibility of those cells having migrated from some other position; the present situation is relieved of such perplexing considerations when Stockard goes so far as to say that the heart, anterior vessels, anterior yolk, and liver are regions in which wandering blood anlagen never make themselves manifest. He also speaks of erythrocytes 'originating' on the yolk when it is certain that their ultimate anlagen did not occupy that position."

Thus it is seen that blood cells are described as 'originating' (64, p. 125) on the yolk, although their anlagen migrated there from some other position. Have we not the same privilege even if it should be demonstrated that cell-migration is responsible for anterior blood cells? Is it reasonable to suppose that erythrocytes should migrate, when in chemically treated embryos which are perfectly normal "so far as the blood anlage is concerned" the leucocytes and lymphocytes of Stockard are *unable to move* from the head regions? The differentiation of a mesenchymal derivative on the teleost yolk-sac in the absence of circulation necessitates migration owing to the original absence of mesenchyme on the yolk. On the other hand, the mesenchyme is so widely distributed throughout the embryo's body that it is not necessary to assume that a blood-producing mesenchyme cell had migrated to a given place within the embryo's body from some other region. Mesenchyme cells undoubtedly can migrate, but the process of migration in case of intraembryonic hematopoiesis would scarcely suggest itself except in support of

a thesis. It has not yet occurred to anyone to claim that the endothelial cells forming in practically all regions of the body have migrated there from other sources or from any single source. If, however, it should be shown that such migration of erythrocyte-forming mesenchyme within the body is a process of importance in the normal development which Stockard's experimental conditions have inhibited, the demonstration of this fact is of importance. Our ultimate aim is an understanding of the normal process. If unfortunately it be true that chemical treatment is not a panacea for our perplexities concerning the normal processes of vascular development, it is desirable that we realize that fact.

Let us now consider certain of the deductions made by Stockard from his results which are said to render the monophyletic view extremely improbable. If my analysis be correct, his entire philosophy of vascular development rests on the following assertion, in favor of which his own work offers no proof. On page 229 (65) he makes this statement which serves as his point of departure: "There can be no doubt of the great genetic difference between blood-cells and connective tissue cells, yet their parent cells are with our present methods indistinguishable." As a matter of fact the doubt which has been entertained and which still exists as to the great genetic difference of blood and connective tissue is the basis of the very problem with which we are concerned. In the next sentence following the one just quoted it is stated: "We may with equal justice go further and hold likewise that the cells from which vascular endothelium, red blood cells and white blood cells arise are mesenchymal cells really differing in nature according to whether they will give rise to one or the other of the three types." This statement is dependent on the one preceding in which we are asked to accept from authority that preformation follows as a matter of course since the end-results are different. On pages 315-316 (65) we have this same idea carried out still farther. Stockard states:

This invisible difference determines the destiny of the cell to form either leucocytes or erythrocytes, but we can not stop just at this point; we must go back to the actual beginning. Then it is found that

although two wandering mesenchyme cells on the yolk of the fish embryo are indistinguishable so far as observations go, yet they are fundamentally different since one is destined to form an erythroblast while the other possesses no such power and can only form an endothelial lining cell or pigment cell as the case may be. This is all the diphyletic or polyphyletic school would ask. . . . Here we logically stop, for this is what is conceived by embryologists to be an anlage. . . . To stop with the tissue anlage we find strong evidence that certain mesenchymal cells are designated to form erythroblasts, others leucoblasts, and still others, and these are more universally scattered throughout the body, give rise to vascular endothelium.

On page 283 (65) Stockard states that the "mesenchymal cell, if taken early enough could no doubt give rise to other mesenchymal cells which would later form these types of cells."

Thus it would seem that our 'conceived' anlage is not a very tangible thing. We could not definitely locate such an anlage and be sure that it might not be a mesenchyme cell which might give rise to other mesenchyme cells 'of these various types.' Its existence is assured by authority and not by demonstration. Some place in the morphologically indifferent stage which we can not locate, 'we find strong evidence' that certain mesenchyme cells are designated to this or that fate. But if it be true that 'here we logically stop' (i.e., looking retrospectively), the converse must be true that looking prospectively, 'here we logically start.' We have selected an instant in which the cell-complex appears by all known means of observation to be indifferent, but our intuition tells us that differences exist. Prior to this time none of the diverse progeny could be said to have an anlage. Now if it be the goal of the polyphyletic school to prove that with actual differences once given, the remainder of the process is, in general, one of greater divergence, their goal is easily reached. Doubtless no one would oppose them. True enough the word 'polyphyletic' etymologically conveys the idea of 'many branches.' If this idea has merely to do with the fate of the parts subsequent to the instant at which we 'logically start,'—if in other words our phyleticism has nothing to do with the origin of the parts beyond the point at which we 'logically stopped,' then the general process of development is uncondi-

tionally polyphyletic. If it were not so, the process of differentiation would never proceed beyond the point at which our intuition tells us that differences exist, though we can not see them. It would seem that "all the polyphyletic school would ask" (65, p. 283) and again that all the polyphyletic school would demand is the privilege of reasoning in a circle.

The attempt has often been made to prove that two tissues could never have come from a common anlage for the reason that when differentiation has taken place the products of differentiation have different appearances. Thus Clark (9) believed himself to have proved that endothelium could not develop from mesenchyme for the reason that the nucleoli and certain other fine structures of the differentiated tissues were different. This finding should not be surprising if the grosser structures themselves were so different as to render possible the distinguishing of the two tissues.⁴

If one were to neglect the transitional stages from mesenchyme into cartilage he might convince himself that the latter could never have come from the former. But should he recognize these transitional stages he might then claim that transformation took place because the transforming mesenchyme cells were destined to that fate and were therefore specific. Yet is there no other alternative? The work of Lewis⁵ and my own work⁶ has shown that in one case at least, preformation need not be assumed; it was shown that otic cartilage arises in response to the presence of a sensory epithelium. The work of Burr⁷ has since shown the same to be true in case of nasal cartilage. Lewis showed that if the otocyst of an anuran be transplanted to the mesenchyme of a urodele, there developed a cartilaginous capsule typically urodelan in character. Here would seem to be a case in which the realized outcome was surely not an expression

⁴ In Prentiss's Text-book of Embryology (Saunders Co., 1915), p. 287, a great deal is made of the fact that F. T. Lewis (Am. Jour. Anat., vol. 5, 1905) observed that "lymphatic spaces do not resemble mesenchyma."

⁵ Lewis, W. H., Anat. Rec., vol. 1, 1907, p. 143.

⁶ Reagan, F. P., Proc. Am. Assn. Anat., Anat. Rec., vol. 9, no. 1.

⁷ Jour. Exp. Zool., vol. 20, no. 2, 1916.

of a predestination. If, on the other hand, otic capsules normally were in the habit of developing in practically all parts of the embryonic mesenchyme, such an experiment would have little significance; in that case, all that could be said is that there would be no especial reason for the belief that the formation of the otic capsule was a matter of predestination; preformation in this instance could neither be proved nor disproved. Suppose for instance that some substance were discovered which would cause the formation of endothelium wherever that substance might be introduced into the mesenchyme. If it be admitted that there are mesenchyme cells in practically all regions which are capable of forming endothelium, it would obviously be impossible to say whether the endothelium which had resulted from the introduction of the substance came from mesenchyme cells whose fate had previously been sealed, or from truly indifferent cells whose vascular characteristics had actually been initiated and not merely unfolded, awakened, or stimulated.

By common consent we have generally agreed that there is no especial reason for believing that a widely diffused mesenchymal derivative is specific. It is generally agreed that before a tissue can be claimed to be specific it must conform to three conditions: First, it must have a demonstrable and narrowly limited anlage whose position we can predict. Second, this tissue must never be found producing any kind of tissue different from the one for which the anlage is claimed to be specific. Third, the anlage producing this tissue must never produce any other kind of tissue. If a mesenchymal derivative fails to conform to any of these conditions, there is no especial reason for believing it to be a 'specific' tissue.

In his final conclusion (65, p. 323) Stockard states:

The facts here presented seem to indicate that vascular endothelium, erythrocytes, and leucocytes, although all arise from mesenchyme, are really polyphyletic in origin: that is, each has a different mesenchymal anlage. To make the meaning absolutely clear, I consider both the origin of the liver and pancreas cells a parallel case; both arise from entoderm but each is formed from a distinctly different entodermal anlage, and if one of these anlagen is destroyed the other is powerless to replace it.

So far as endothelium is concerned, the two cases are most certainly not parallel. There is abundant evidence for the belief that endothelium can form locally in the mesenchyme in practically all parts of the germ layer of which it is a constituent. There is no evidence that pancreatic cells form locally in, or even migrate into practically all regions of the entodermal tissue. One can predict, in case of pancreatic and liver tissues, approximately the very region in which the anlagen will make themselves manifest; to say the very least, one can predict that in the greater part of the entoderm no pancreatic or liver anlagen ever exist. This can not be said to be true of mesenchyme cells capable of forming endothelium. If the results outlined in my own work hold good for teleost blood-cells, the development of the latter will likewise be found not to constitute a case comparable to that of the development of pancreas and liver.

The inability of one differentiated tissue to replace or regenerate another has absolutely no bearing on the question. In some species a given organ can be regenerated while in other closely related species that very same organ can not. Obviously a comparison of the regenerative power of different tissues of the same organism or of their ability to replace each other has no importance for the question at hand. The ordinary process is not for a destroyed tissue to be replaced by another equally differentiated tissue, but to be regenerated by a tissue intermediate between the two and more primitive than either.

The monophyletic view does not hold that development shall be chaotic and without system. For the monophyletic view it is not necessary that the diverse mesenchymal derivatives should invariably be found in close association. If it be true that there is really an equipotential stage in which the fate of a mesenchyme cell is determined in part by factors extraneous to its own constitution, it does not necessarily follow that the factors which direct the development of these cells into different avenues will all operate at the same place or at the same time in that truly equipotential system. Neither is the possibility precluded that in some parts this equipotentiality or embryonic condition may persist until a late stage in the ontogeny, making possible a plasticity of interadaptation and a regulation of parts

—a phenomenon long since recognized and known as indeterminate development. The great importance and great complexity of the intercellular environment is little appreciated. We are likely to consider this type of environmental differences as of little importance for the reason that we know little about them.

Again I wish to state as I have twice done previously that “the development of the vascular system furnishes an unproductive field for the solution of the problems of preformation and epigenesis.” A diffuse tissue like the mesenchyme is the very worst sort of tissue in which to prove predestination by authority.

Despite the very important work which has recently been done on the vascular system, the interest which attaches to its main problems is by no means merely historic. The vascular system still may have some surprises in store for us. The fact that the problem can be attacked experimentally need by no means belittle its importance. As yet we are still in the stage of testing our methods, and will do well to accept with caution the results of experimental methods, remembering that experimental conditions may obscure the normal process. The very best we can do experimentally is to subject the embryo to as many methods of experiment as possible, selecting results from those methods which alter to the least extent the normal process. By combining the pictures obtained from these various methods into a composite picture we may by chance obtain a resultant which will be of service to us in the study of the normal process. By determining as many as possible of the things which a tissue is able to do, we obtain information as to what it does do. We may, perchance, sometimes find that a given tissue can do some of those things which it had previously been regarded as unable to do; this alone would justify experimental effort.

I wish to make clear that in no case do I regard an experimental condition as portraying a truly normal process, and have therefore not volunteered an estimate of the significance to be attached to the observed conditions in each experimental case. It is too much to dare hope that my results will meet with ready acceptance; all I can say is that during the time in which I have worked, I have spared neither labor nor pains to make these results accurate.

III. SUMMARY

The foregoing results furnish evidence in favor of the following propositions:

First, that mesenchyme in many regions of the body can turn into endothelium, and that endothelium is not an ingrowth from vessels on the yolk.

Second, that prevascular tissue can come from more than one germ-layer.

Third, that mesenchyme cells which can form a given type of blood cell are not confined to a narrowly limited region of the embryo.

Fourth, that endothelium can transform into blood-cells.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

11 Section through the anterior portion of the forebrain of a head-meroplast, showing unusual head-coelom. Total incubation thirty-two hours. Operation at the time of the first intersomitic groove ($\times 160$). Experiment, Type I, no. 19; *b*, anlage of ventral aorta; *c*, coelom; *d*, pharynx; *e*, forebrain.

12 Section through the forebrain of a head-meroplast, showing early stages in the formation of vasofactive cells. Total incubation, twenty-nine hours: Operation previous to the formation of the first intersomitic groove ($\times 200$). Experiment, Type I, no. 24; *a*, prevascular mesenchyme; *c*, coelom; *d*, pharynx; *e*, forebrain.

13 Section through the forebrain of a head-meroplast showing a loose parenchyma in a position occupied by the isolated vasofactive cells of figure 4. Total incubation thirty hours. Operation at the time of the first intersomitic groove ($\times 150$). Experiment, Type I, no. 18; *a*, prevascular mesenchyme; *c*, coelom; *d*, pharynx; *e*, forebrain.

PLATE 2

EXPLANATION OF FIGURES

14 Section through the forebrain of a head-meroplast showing anlagen of the ventral aorta. The longitudinal incision on the right was relatively close to the neural fold. The cut edges of the pharyngeal entoderm have pulled apart the ventral tissues having swung to the left. Experiment, Type I, no. 31 ($\times 240$). *b*, ventral aorta; *c*, coelom; *d*, pharynx; *e*, forebrain.

15 Section through the forebrain of a head-meroplast showing a well-defined ventral aorta. The right longitudinal incision was close to the neural fold. None of the excised head has regenerated; the mesenchyme is very compact near the cut surface the cells of which are somewhat epithelial. Total incubation thirty-three hours. Operation at the time of the first intersomitic groove ($\times 175$). Type I, no. 17; *b*, ventral aorta; *c*, coelom; *d*, pharynx; *e*, forebrain.

16 Section through the forebrain of a head-meroplast showing a proamniotic sac containing a pouch of coelomic mesothelium. On the ventral side of the sac is a peculiar proliferation of entodermal cells very constantly appearing in experiments of this type, generally more symmetrically situated. Total incubation, forty-eight hours. Operation at the time of the second intersomitic groove ($\times 55$). Experiment, Type II, no. 3; *c*, coelom; *e*, forebrain; *f*, ectoderm; *g*, entoderm; *j*, extra-embryonic vessels.

PLATE 3

EXPLANATION OF FIGURES

17 Photograph of a section through the midbrain of the same microplast as in figure 16, showing well developed dorsal aortic and the absence of a tubular pharynx in a tubular head. Fusion of entoderm and ectoderm at points indicated by *x* ($\times 120$). *f*, ectoderm; *g*, entoderm; *h*, dorsal aorta; *i*, midbrain.

18 Photograph of one of the first available sections of the blastoderm behind the incision *G* to *H* of Experiment Type II, no. 3, showing the freedom of the pellucid area from endothelium ($\times 160$).

19 Photograph of a transverse section of the removed blastoderm of Experiment Type I, no. 31. One of the first available sections behind the transverse incisions, showing especially well the position of the longitudinal incisions and the status of the extraembryonic vascular tissue. *Endth.*, endothelium; *Pv.mch.*, prevascular mesenchyme.

20 Photograph of the removed blastoderm of Experiment Type I, no. 122, showing the absence of all vascular tissue at the time of operation (prior to somite-formation). See also figure 22.

PLATE 4

EXPLANATION OF FIGURES

21 Portion of a transverse section through the pharyngeal region of mero-plast Type I, no. 31, near the plane of figure 14.

22 Portion of a transverse section through the pharyngeal region of mero-plast Type I, no. 122, showing conditions very similar to those in figure 21. The endothelial tubes of the two sides are in communication ventral to the pharynx. The endothelium stains more darkly than does the mesenchyme.

ABBREVIATIONS

Coel., coelom

Endth., endothelium

Ent., entoderm

Par. mes., parietal mesoderm

Vis. mes., visceral mesoderm

PLATE 5

EXPLANATION OF FIGURES

23 Section through the posterior heart-region of meroplast Type II, no. 133.

24 Transverse section of meroplast Type II, no. 96. The unilateral heart of this embryo communicates with extraembryonic vessels. The aortic anlagen are discontinuous.

25 Transverse section through the trunk of meroplast Type II, no. 116, showing a beginning of splanchnopleural conerescence.

26 Section more anterior in same meroplast showing apposition of conerescing entoderm to close off the pharynx.

ABBREVIATIONS

Aort., aorta

Coel., coelom

Ect., ectoderm

Endc., endocardium

Endth., endothelium

Ent., entoderm

Myc., myocardium

Ot. ep., otic epithelium

Par. mes., parietal mesoderm

Ph. pharynx

Vis. mes., visceral mesoderm

PLATE 6

EXPLANATION OF FIGURES

27 Section through the heart of the same meroplast showing completion of ventral 'body wall' and remains of ventral mesentery of the heart. Note the well developed endocardium and dorsal aortae.

28 Section through the fore-gut of meroplast Type II, no. 52, showing numerous endothelial cysts and mesothelial folds. The entire body wall is covered with ectoderm. Section is slightly frontal.

29 Transverse section through the multiple unilateral heart anlage of meroplast Type I, no. 111.

30 Transverse section through the posterior axial region of meroplast. Type II, no. 124, showing unequally developed dorsal aortae.

ABBREVIATIONS

Aort., aorta

Ect., ectoderm

Endc., endocardium

Endth., endothelium

Ent., entoderm

Myc., myocardium

Par. mes., parietal mesoderm

Ph., pharynx

Pv. mch., prevascular mesenchyme

Vis. mes., visceral mesoderm

x, point of fusion of ectoderm and entoderm

PLATE 7

EXPLANATION OF FIGURES

31 Section through the posterior axis, Type II, no. 149, showing a small aortic anlage which has developed in a region devoid of a coelom.

32 Section through an early visceral mesodermal proliferation of pre-endothelial tissue in the aortic line anterior to the plane of figure 31.

33 Section between the planes of figures 31 and 32 showing a ventrally directed proliferation of visceral mesoderm in the aortic line.

34 Transverse section through the posterior axial region (Type II, no. 53) showing small dorsal aortae.

35 Transverse section through the heart region of Type I, no. 34, showing endocardium approximated to entoderm.

36 Transverse section through anterior heart region of same meroplast showing endothelium apparently being delaminated from mesoderm.

ABBREVIATIONS

Aort., aorta

Endc., endocardium

Ent., entoderm

Pv. mch., prevascular mesenchyme

Vis. mes., visceral mesoderm

x, point of fusion of ectoderm and entoderm

PLATE 8

EXPLANATION OF FIGURES

37 Transverse section through trunk of meroplast Type II, no. 88, showing the proliferation of endothelium by visceral and by parietal mesoderm.

38 Section posterior to the plane of figure 37, and more highly magnified.

ABBREVIATIONS

Ect., ectoderm

Endth., endothelium

Ent., entoderm

Pv. mch., prevascular mesenchyme

PLATE 9

EXPLANATION OF FIGURES

39 Transverse section through the heart-region of meroplast Type II, no. 14. On the right side of the figure will be noted a thickening of the entoderm.

40 Portion of the same section more highly magnified. The mass of cells lying against the entoderm has evidently been proliferated by that tissue.

ABBREVIATIONS

Ent., entoderm

Par. mes., parietal mesoderm

Pv.m., prevascular mesoderm

Vis. mes., visceral mesoderm

x, point of fusion of ectoderm and entoderm

PLATE 10

EXPLANATION OF FIGURES

41 Section slightly anterior to the plane of figure 40, showing the entodermal proliferation slightly separated from the entoderm.

42 Section anterior to the plane of figure 41, one portion of the entodermal proliferation still in contact with the entoderm.

PLATE 11

EXPLANATION OF FIGURES

43 Section much anterior to the plane of figure 42, showing a double endothelial tube surrounded by myocardium. This endothelium can be traced back continuously to solid tissue the base of which is shown in figure 40.

44 Transverse section of the middle heart region of meroplast Type II, no. 48.

ABBREVIATIONS

Endc., endocardium
Endth., endothelium
Ent., entoderm

Myc., myocardium
Par. mes., parietal mesoderm
Vis. mes., visceral mesoderm

PLATE 12

EXPLANATION OF FIGURES

45 Section through the anterior heart region of the same meroplast showing an entodermal proliferation of prevascular mesoderm

46 Section between the planes of figures 44 and 45 showing a connection of endothelium with entoderm.

47 Section through the posterior heart region of the same meroplast showing endothelium connected with entoderm.

48 Section posterior to the plane of figure showing the prevascular tissue in connection both with entoderm and with mesoderm. The connections with the latter are not mere coagulations of a clear plasma, but are composed of a real intercellular matrix.

ABBREVIATIONS

Endth., endothelium

Ent., entoderm

Pv. mch., prevascular mesenchyme

PLATE 13

EXPLANATION OF FIGURES

49 to 57 Dorsal views of the cephalic portions of chemically treated embryos. Figures 57 and 58 are lateral views. The stippled areas in these figures indicate the location of erythrocytes as observed in the living embryo.

49, 50, 53, 55 Represent the heads of fourteen-day embryos, which at their four-cell stage were treated for twenty-four hours with a solution of 50 cc. sea-water to which had been added 15 cc. of M/12 butyric acid. They are all from the same experiment, one-fourth of the embryos in which yielded similar results. Owing to the evanescence of the red coloring, it is possible that many others may have passed through this critical stage without being observed.

51 and 52 Described with sufficient detail on page 95.

56 and 57 From embryos obtained from the same experiment as that of figures 43 and 44.

58 From an embryo, the treatment of which is described on page 95. *Erth.*, erythrocytes; *Ht.*, heart.

59 Represents an embryo, only a small portion of the body axis of which developed. No heart formation has taken place. There is a large dilated pericardium in the region of which are many isolated erythrocytes. Embryo from same experiment as that from which the embryos of figures 49, 50, 53 and 55 were obtained. It seems improbable that the 'pericardium' of this embryo should be interpreted as Kupffer's vesicle. Even so, there are blood cells of local origin on what would then be the anterior yolk, not shown in this figure.

60 Represents a case in which the embryonic body has failed to develop, or as such has been transformed into mesodermal and vascular tissues which are scattered entirely over the yolk. The embryo at the four-cell stage was treated for ninety-six hours with a solution of 50 cc. of sea-water to which was added 15 cc. of a molecular solution of acetone.

61 A lateral view of a twelve-day embryo treated for twenty-four hours subsequent to the four-cell stage with a solution similar to that employed in case of the embryos represented by figures 49, 50, 53, 55 and 59. Kindness of Dr. E. I. Werber.

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PLATE 14

EXPLANATION OF FIGURES

62 A transverse section through the optic region of a nine-day embryo which at the four-cell stage was treated with a solution of 50 cc. sea-water, to which had been added 15 cc. M/12 butyric acid. The yolk-sac is devoid of vascular tissue. The space in the optic cup (on the left side of the figure) normally occupied by a lens, contains a blood lacuna. On the right side is seen the attachment of the upper optic stalk and the base of the lower.

63 Transverse section of the same embryo from which figure 62 is taken; it is a section passing through the optic anlagen, showing isolated erythrocytes, solid heart, and unusual eye-formation.

ABBREVIATIONS

Bl.l., blood lacuna
Endc., endocardium
Erth., erythrocytes
Ht., heart
L., lens

Myc., myocardium
Oc., optic stalk
O.st., optic stalk
Y.s., yolk sac

PLATE 15

EXPLANATION OF FIGURES

64 Section of the same embryo slightly posterior to the optic stalks, showing the precardinals as haemophoric vessels. The erythrocytes are angular in contour (especially in the vessel on the right of this figure) due to their crowded condition. A few of the erythrocytes in the less crowded vessel on the left have become rounded.

65, 66, 67 and 68 Transverse sections of the embryo from which figure 49 was drawn.

65 Section just posterior to the optic cups passing through the anterior ends of the haematopoietic areas of this region as shown in figure 49. In this figure, as in figure 66, the position of the blood-cells is roughly and arbitrarily indicated by heavy black dots.

66 Section passing through the posterior portions of the optic cups, and showing the position of the median blood anlage of figure 49. The heart in this figure is a solid continuation of the left portion of the double body labeled heart in figure 65.

67 An accurate detail of the blood-producing areas seen on the right side of the head in figure 65. Conditions in the lower lacuna seem to be farther advanced. Certain mesenchyme cells (lightly stippled) are in a stage of transition to erythrocytes (cytoplasm clear). A blood space is forming while the mesenchyme cells bounding it seem to be receding to form endothelium. The free erythrocytes are rounded in contour.

68 An accurate detail of the median dorsal blood anlage of figure 66. The blood-cells are very strongly eosinophilous but are crowded and possess angular contours.

ABBREVIATIONS

Erth., erythrocytes

Ht., heart

PLATE 16

EXPLANATION OF FIGURES

69 and 70 Are described in detail on page 96. The small arrows indicate the direction of pulsation in the 'accessory heart.' Note the apparently normal condition of the actual heart.

71 Section through the heart of this same embryo near the plane of section of figure 65. The connection of the fused portions as described in the text is looser than in the plane of figure 65. The right 'arterial' side contains no endothelium in this section. Erythrocytes in various phases of development are observed. Note the cuboidal nature of the endocardium in the left side of the figure.

72 Section through the heart of the embryo from which figure 55 is made. In this figure the endocardial cells are cuboidal and rather deeply staining. The endocardial cavity contains erythrocytes which are in a column continuous with the erythrocytes of figures 73 and 74. There is a more or less distinct myocardium.

73 Shows a section of the same heart in which a portion of the endocardium and myocardium are transforming into eosinophilous blood-cells.

74 From the same heart. The plane of section is near the upper end of the heart. The entire endocardium and myocardium of this region has transformed into eosinophilous blood-cells which, as in many instances already noted, are unable to round themselves out for lack of space.

ABBREVIATIONS

A.ht., accessory heart
BL., blood lacuna

Endc., endocardium
Erth., erythrocytes
Myc., myocardium

PLATE 17

EXPLANATION OF FIGURES

75 Section through the otocysts of the embryo of which figures 62, 63 and 64 are sections. The precardinal lines are indicated by erythrocytes. There are erythrocytes in the very rudimentary short ventral aorta.

76 Section through the optic region of an embryo of *Erimyzon sucetta oblongus*. Embryo at cell stage was treated with a weak solution of KCN. Dorsal to each eye are erythrocytes in all stages of development.—Kindness of Professor McClure.

ABBREVIATIONS

Erthbl., erythroblast

Erth., erythrocyte

PLATE 18

EXPLANATION OF FIGURES

77 Section through the heart of the same embryo (see fig. 76) showing the solidity of the proximal portion of the heart and the almost complete absence of anterior vessels.

78 Section tangential to the posterior surfaces of the eyes showing the absence of endothelium posterior to the groups of erythrocytes seen in figure 76.

79 Section through the nasal sensory epithelia of the same embryo showing a group of erythrocytes in an endothelial cavity near the sensilla of the left side.

80 Section through the liver of this same embryo showing erythrocytes within the liver tissue and their absence in the aorta.

ABBREVIATIONS

Erth., erythrocytes
Ht., heart

Lvr., liver
Nas. ep., nasal epithelium

PLATE 19

EXPLANATION OF FIGURES

81 Section through the liver of the embryo from which figures 62, 63, 64, and 75 are made. Erythrocytes are seen in various stages of formation from mesenchyme cells.

ABBREVIATIONS

Erthbl., erythroblasts

Erth., erythrocytes

PLATE 20

EXPLANATION OF FIGURES

82 Total view of a living cardiectomized embryo of *Fundulus*. Age nine days. Operation at seventieth hour. Column of erythrocytes can be seen through otocyst. Erythrocytes and endothelium present on anterior yolk but absent on posterior yolk. Eyes very defective.

83 Transverse section through the otocysts of the embryo represented in figure 82. Erythrocytes indicate precardinal lines. *Erth.*, erythrocytes.

PLATE 21

EXPLANATION OF FIGURES

84 A living ten-day *Fundulus* embryo in which the heart anlage was destroyed at the 60-hour stage. Erythrocytes developed anterior to the pectoral fins, on the anterior yolk just ventral to the pericardium, and in a peculiar position dorsal to the posterior yolk, as well as in the intermediate cell mass and on the posterior yolk.

85 A living twelve-day *Fundulus* embryo in which the heart anlage was destroyed at the 60-hour stage. At the same time the head was severed. A large 'pericardial' cavity has formed. Erythrocytes formed between the otocysts, in the ducts of Cuvier, and in the intermediate cell mass.

PLATE 22

EXPLANATION OF FIGURES

86 Section through the yolk sac of a chemically treated embryo showing the position of a transplanted cephalic meroplast.

87 The same section of the meroplast more highly magnified showing an otic capsule, and mesenchyme devoid of erythrocytes.

88 A section through the anterior-most region of the meroplast showing the axial portion to be solid cartilage.

89 Section between the planes of figures 87 and 88, more highly magnified showing mesenchyme cells in various stages of transition into erythrocytes.

ABBREVIATIONS

Cart., cartilage

E., eye

Erth., erythrocytes

Erthbl., erythroblasts

Gng., mass of ganglionic tissue

Ot.c., otic capsule

Otcsl., otocyst

Y.s., yolk sac