XIX.—On the Histology of the Blood of the Larva of *Lepidosiren paradoxa*.
Part II. Hæmatogenesis. By Thomas H. Bryce, M.A., M.D. (With Four Plates.)

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INTRODUCTION.

In the first part of this memoir I described the structure of the corpuscles at a stage of larval development when the red cells were actively dividing and the blood contained several varieties of white cells. During the course of these more strictly cytological observations, it was impressed upon me that the great size of the elements and their very marked histological characters, combined with the simple character of the organisation of the animal, made *Lepidosiren* a very favourable case for the study of the first principles of Hæmatogenesis. I was specially interested in what may be termed a middle phase in the history of the blood. I refer to a period after the primitive corpuscles have acquired hæmoglobin and there are leucocytes present, but before the blood-forming organs are unfolded. This stage lasts a relatively long time in *Lepidosiren* up to the differentiation of the spleen, as the liver takes no part in blood-formation at any period.

Since BIZZAZZO first discovered that hæmoglobin-containing cells divide by mitosis, and emitted the hypothesis that the red cells are a stirp kept up only by division, it has been largely held that all forms of the coloured corpuscles are descendants of those first laid down.

The admission of a non-hæmoglobin-containing element into the erythrocyte series (LOWIT and DENYS) only pushed back the argument from the 'hæmatoblast' of NEUMANN to the 'erythroblast' of LOWIT. If the erythrocyte series constitutes a 'tissue' *sui generis*, when does it cease to be laid down?

We know from observations on the characters of the corpuscles at various stages of development that in all vertebrates the elements are at first identical, but that their characters change, and the adult corpuscles are different in character from those which first appear. It is sometimes assumed that the adult nucleated erythrocytes of the lower vertebrates correspond to the nucleated discs of mammals, but in the *Lepidosiren* larva the erythrocyte series shows all the stages seen in the higher forms. There are 'erythroblasts' without hæmoglobin; 'hæmatoblasts' with hæmoglobin; young erythrocytes with reticular nucleus; and mature or old forms with vesicular nuclei. All forms save the last divide by mitosis, and the last is probably only a hæmoglobin carrier like the mammalian red blood corpuscles.* Thus, though the outward form of

* Cf. LAGUESSE, *Journal de l'Anat.*, T. 26, 1890

the elements is rather different, the *essential* problem is the same in both lower and higher animals. The heterogeneity of the erythrocyte series through the whole larval life, and the fact that all the elements except the mature erythrocyte multiply by mitosis and after their kind, seems a presumption in favour of continued new formation. If this were so the erythroblasts would necessarily bear a relation to some less specialised cells. Analogous facts in other forms have led to the theory, in all its varieties, of the origin of the red cells from the white elements.

From the theory that the vascular and lymphatic systems are formed in the mesenchyme (ZIEGLER), whatever view may be taken of the actual channels and spaces, it follows that the cells, fixed and free, red and white, are all homologous, having the same parentage. Further, the theory gives room for the acceptance of continuous formation of free cellular elements, at any rate up to the completion of histological differentiation, and of the genetic relation in some sort between the red and white corpuscles.

On the other hand, if the vessels arise *in situ* but the blood has a restricted local origin in one part of the embryo from which the corpuscles are dispersed—as pointed to by the works of RÜCKERT, RABL, ZWINCK, SWAEN and BRACHET—and the sites of origin are not also those of the germs of the connective tissue, the blood and the lymph may, from the embryological and morphological point of view, require to be distinguished (FELIX, SWAEN and BRACHET). The two classes of corpuscles might then belong to two separate stirps, multiplying by division and having no mutual relationship.

The origin of the first leucocytes has not yet been demonstrated beyond doubt, but the almost universal opinion has referred them to the mesoderm (mesenchyme). This is ZIEGLER's* view, and in this country it has been specially maintained by GULLAND. They are 'wandering cells' formed outside the blood stream; and as they appear at a later stage of ontogeny than the primitive blood corpuscles, they belong to a different category.

In recent years, however, there has been a tendency to derive the lymphoid cells direct from the endoderm. KÖLLIKER first described the epithelial cells of the thymus as becoming converted into lymphoid cells; PRENANT, SCHULTZE, MAURER, NUSSBAUM and PRYMAK, and BEARD† have come to a similar conclusion, and the last named claims for the gland that it is the sole source of the leucocytes.

A similar conversion of the epithelial cells of the gut into the lymphoid cells of the intestinal glands has been described by RETTERER, RUDINGER, DAVIDOFF, and KLAATSCH, but it has been denied by STOHR and KOLLMANN.‡

In the matter of the spleen there has also been a question of the endoderm providing the cells of its rudiment. This view has been put forward specially by MAURER and KUPFFER, but the older view that it is formed from the mesoderm as a mass of

* *Ber. der Naturforsch. Gesellsch. zu Freiburg*, Bd. iv., 1889. *Verhandl. d. deutschen Zool. Gesellsch.*, 1892.

† *Zool. Jahrbucher Abt. f. Anat.*, vol. 17, 1903. For a historical account of histogenesis of thymus and references to literature this work may be consulted.

‡ For critical review and references, see KOLLMANN, *Archiv f. Anat.*, 1900.

mesenchymatous cells, maintained by LAGUESSE, has been upheld by most of the recent workers, especially by TONKOFF, KOLLMANN, KEIBEL, and PIPER.*

My observations on the blood of *Lepidosiren* have not included the first origin of the blood and vessels, but have been directed specially on the later phases, for the study of which *Lepidosiren* presents very favourable opportunities. I have, however, made an exhaustive examination of the blood corpuscles of all the early stages, so that my observations extend over the whole history of the blood, from the time the first corpuscles appear in the heart onwards. I have necessarily studied the histogenesis of the spleen, and the development of the lymphoid tissue, so called, in the kidney, and have made incidental observations on the thymus gland. On this last head I shall have little to say, as I hope soon to study the development of the gland in detail.

For the purpose of the research, I have had the opportunity, through the kindness of my friend Professor GRAHAM KERR, of going through the whole of the larval stages. This has involved the exhaustive examination of more than twenty series—up to stage No. 38 of the sequence, when the larva is already practically a small adult *Lepidosiren*. I have also examined sections of the modified filamentous hind limb of the male during the degeneration which ensues after the breeding season, when the tissues are crowded with leucocytes.

As the result of these more extensive observations, I have not much to add in regard to the structure of the fully evolved elements.

The erythrocytes in the adult and later larval stages have almost invariably a vesicular nucleus, and the few corpuscles that have a nucleus with the coarse reticulum of the earlier phases are to be regarded as young erythrocytes. The equatorial band is less distinct in the adult corpuscles, and the reticular structure of the disc is more doubtful. The adult material is unfortunately not sufficiently well fixed to enable me to determine whether the reticular appearance of the adult corpuscle is wholly an artifact or not; but as the granular, irregularly reticular appearance of the disc closely resembles that of the reticulum of the early corpuscles in a badly fixed condition, I think it probable that the larval and adult elements resemble one another in this respect also. I make, however, the same reservations in regard to this structural feature as I did in my previous communication. I find that the leucocytes of the adult and late larval stages present no essential differences from those of the earlier stages described in Part I. I must add to that account, however, that I find forms with basophile granules. The cells containing these do not differ in general character from those with eosinophile granules, and, as in them, the granulation is either fine or coarse. I shall reserve what I have to say on the general morphology of the leucocytes until I have described their development.

Before proceeding to the record of my observations, I must refer to certain general points.

* Complete historical accounts are given by CHORONSHITZKY, *Anat. Hefte*, Bd. 13, 1900; and PIPER, *Diss. Med.*, Freiburg, 1902. See also specially KOLLMANN, *Archiv f. Anat.*, 1900.

A description of the external characters of the embryo and larva of *Lepidosiren* and a general account of the affinities of the animal will be found in Professor GRAHAM KERR's original memoir in the *Transactions* of the Royal Society, B., vol. cxcii., and an account of the early stages of development in his paper in the *Quarterly Journal of Microscopical Science*, vol. xlv. part i.

The egg is markedly telolecithal, but the segmentation is holoblastic. The excessive lading of the cells with yolk, and the consequent large dimensions of the primitive yolk cells, impresses characters on the development remarkable in certain respects. Among the secondary features due to this cause, the one that chiefly affects the present study is the long postponement of the formation of the alimentary canal. Even at the stage numbered 31, the pharynx, gill clefts, and alimentary tract are solid, showing in no part an epithelial disposition of the cells. As a further consequence of this, the splanchnic mesenchyme is long in being differentiated. The splanchnic mesepithelium rests on a layer of smaller yolk-laden cells, in which blood-vessels form, but which is not separate from, or to be distinguished from, the general mass of the yolk cells. It is only when the alimentary canal commences to be differentiated that a layer of mesenchyme and a layer of definitive epithelial hypoblast cells can be distinguished.

The general mesenchyme is differentiated much earlier. It arises, GRAHAM KERR states, for the most part by a proliferation from the mesoderm, at about the level of the nephric rudiment, very much as in the Selachians, partly directly from the sub-notochordal region of the hypoblast.

The history of the blood corpuscles may be divided into three phases. The *first* phase extends over the period from their origin up to stage 30, when the alimentary canal commences to be cut off. It is coincident with the laying down of the heart and main vessels. While the phase lasts, the corpuscles, like the rest of the tissue cells, are laden with yolk; and though they vary much in character according to the amount of yolk borne, are at first all of one type, though very soon two kinds are to be distinguished.

The *second* phase is coincident with the formation of the alimentary canal and the differentiation of the splanchnic mesenchyme. The blood corpuscles, now free of yolk, like all the tissue cells save the hypoblast, are heterogeneous. There are erythroblasts, young erythrocytes in active division, and mature red cells, while, further, there are white elements belonging to different categories. The rudiment of the spleen appears at the beginning of this phase, and during its persistence is undergoing its histogenetic changes.

As the phase advances the erythrocytes become almost all of the mature variety, and the *third* phase is initiated, during which the permanent conditions are established. The great mass of the red cells are now mature, young corpuscles are sparsely distributed, while the erythroblasts seen here and there in the vessels are densely crowded in the spleen pulp, and to a lesser degree in the venous sinuses of the kidney

(mesonephros). The leucocytes, in their several varieties, are coursing in the blood stream or wandering among the tissues, especially in the walls of the alimentary tract; they are also crowded in the spleen and, as will afterwards be shown, along a tract around the kidney.

Though I have thus divided the history of the blood corpuscles into three phases, it is not to be understood that they are sharply marked off from one another, but that they overlap and merge into one another imperceptibly.

PHASE I.

The Primitive Blood Corpuscles.

With the actual primary origin of the blood corpuscles, and the development of the heart and vessels, this paper will not concern itself, nor will it deal with the general question regarding the morphological relations of the blood in terms of the germ layers. This will be dealt with by Mr GRAHAM KERR himself in a future memoir.

I begin at a stage, No. 26, when the rudiment of the heart is laid down. In it there are free cells, not to be distinguished by any of their characters from the fixed cells forming its wall. In the middle line, below the notochord, the aorta appears like a cord of cells, identical in general characters with the cells of the developing general mesenchymatous tissue. The same is true for the cardinal vein.

On the surface of the mass of yolk cells, immediately beneath the fused somatic and splanchnic layers of mesepithelium, there are groups of free rounded cells, in spaces which later become definite vessels (fig. 1, Pl. I.).

As I have said, I have not actually studied the origin of these various free rounded cells, but from certain incidental observations, and from later phases of the development of the corpuscles, I may express my belief, subject to the revision of Mr GRAHAM KERR's future researches, that the corpuscles have a multiple origin *in situ* from the general mesenchyme in connection with the developing blood-vessels, and from an irregular layer of smaller yolk-laden cells lying beneath the splanchnic mesepithelium, on the exact provenance of which I do not wish to express an opinion. I make this statement with every reserve, and only make it at all because of its bearing on later stages.

The characters of the primitive blood corpuscles (figs. 1 and 2, Pl. I.) are determined by the size and number of the yolk masses in the protoplasm. In fig. 2 is represented at one pole of the nucleus an area free of yolk, including a spot I have taken for the centrosome, and from it, radiating among the yolk grains, there are delicate threads of protoplasm reaching the periphery of the cell. The nucleus is rounded or slightly oval, and sometimes shows a notch. The chromatin is collected into rounded karyosomes, from which delicate processes ramify to join those of other karyosomes to complete the reticulum.

In a stage older, No. 27, the appearance of the corpuscles is considerably altered

This is due in some measure to different methods of treatment, the earlier stages being celloidin sections stained with safranin, while these are paraffin preparations stained with iron hæmatoxylin.

Two kinds of corpuscles are to be recognised. The first, evidently the direct derivatives of the corpuscles of the previous stage, have a large round or slightly oval body, measuring $50\ \mu$ to $60\ \mu$, and a round nucleus which has the same general features as described for that of the earlier elements.

The protoplasm round the periphery of the cells has undergone a transformation into a broad band of delicate concentric fibrils. Within this the protoplasm has a delicate alveolar structure and contains large yolk grains, and in some corpuscles clear vacuoles. In the particular cell drawn (fig. 3, Pl. I.) there was a homogeneous area at one pole of the nucleus which I take to represent the centrosomal area of the earlier corpuscles, but there is no distinct centrosome, nor is there a radial sphere. Most of these cells are round, but a few are oval in shape (fig. 4, Pl. I.).* The cell of this class drawn has only a few yolk grains, the alveolar disposition of the protoplasm is distinct, and at each end of the elongated body there is a group of minute darker-staining granules, which I interpret as the cross sections of the peripheral fibrillæ of the other cells, and which, as will appear later, in more advanced corpuscles come to occupy this situation in profile sections.

The second kind of corpuscles are smaller cells, about $39\ \mu$ in diameter (figs. 5 and 6, Pl. I.). The nucleus is notched or lobed, but has otherwise the same general characters as that of the larger corpuscles. The protoplasm is either uniformly alveolar or vacuolated. There is a distinct sphere and centrosome, and the peripheral band is absent.

The vacuolation of the corpuscles at this and later stages is evidently connected with the using up of the yolk. It is seen in the general tissue cells also. The fibrillar band is seen only in the blood corpuscles, and is clearly the first stage in the conversion of the primitive blood corpuscles into the passive hæmoglobin carriers of later phases.

It seems to be a fibrillar transformation of the peripheral layers of the protoplasm, and not a mere disposition of an alveolar meshwork; and as none of the corpuscles have yet the biconvex disc shape, it is not the mere consequence of the shape of the cell.

While the mass of the corpuscles are thus assuming a passive rôle, certain remain as free mobile elements, constituting the second type of cell described, with its sphere and centrosome, and the lobing of its nucleus somehow associated with the activities of the cell.

While one cannot at this stage, when there are yet no hæmoglobin-bearing cells, call these elements leucocytes, it is to be noticed that in general morphological

* It is probable that these are profile sections of the circular disc-shaped forms.

characters they are identical with the later leucocytes. I may also remark that the general mesenchyme cells show in certain instances all the characters of these free cells in the blood, and that some even have definitely polymorphic nuclei.

The next three stages may be described together, as there is no marked difference in the general characters of the blood. They are numbered 28, 29, and 30.

The large ringed corpuscles have increased greatly in number, partly by active division, partly by new formation. That new formation is proceeding, I conclude, because both in the earlier stages and in those now in review there are appearances in the developing vessels under the mesepithelium over the mass of undifferentiated yolk cells which indicate the budding off of free elements from the superficial layer of smaller yolk-laden cells, and it seems to me probable that the opening out of the cardinal veins in the mesenchyme round the nephric ducts is also associated with the setting free of elements into the blood stream. The enormous amount of yolk in all the cells at this stage, and the consequent large size of the cells (each cut through, in $10\ \mu$ sections, at least three times), make it very difficult, however, for one to satisfy one's mind regarding this.

Some of the yolk-laden blood corpuscles are at this stage of enormous dimensions. These giant corpuscles (fig. 6, Pl. VII.) are either round or oval. The round cells are about $60\ \mu$ in diameter; the ring is very broad and distinct, enclosing the yolk grains and vacuolated protoplasm, from which it is sharply marked off. The large oval cells (fig. 7, Pl. I.) attain a length of $80\ \mu$ to $90\ \mu$; I have met with individuals even more than $100\ \mu$ in length. In the smaller cells of earlier stages, as far as I can make out, the layer of superficial fibrillar protoplasm surrounds the greater part of the corpuscle, but in the elongated corpuscles it is massed at the ends, *i.e.* at the equator of the oval disc. The nucleus of all the corpuscles is of the 'leucoblast' type. It is spherical in the great majority of these large cells, but numbers have lobed or even multiple nuclei, and these often of very unequal size (fig. 11, Pl. I.). In the earlier stages of these three series under consideration mitotic figures are numerous; in the later they also occur, but less frequently, and the question arises whether the multiple nuclei arise by direct or indirect division.

I have little doubt in referring to direct division certain cases in which the nucleus is a regularly shaped dumb-bell, and in explaining them as antecedent stages of cells with two equal nuclei lying side by side. In other cells of a smaller variety, to be mentioned immediately, the nucleus is irregularly lobed, and there are sometimes detached free portions, which must be produced by direct fragmentation. On the other hand, certain instances may be the result of indirect division of the nucleus without division of the yolk-laden body. The mitotic figures are not numerous enough to enable me to obtain a complete series without much labour, so that I have not followed this point out to a judgment. For the same reason I have been unable to determine the relation, if any, to the multiple and unequal condition of the nuclei, of a number of instances observed of irregular mitoses, some of which were multipolar.

Up to this stage none of the corpuscles contain hæmoglobin, as I have been able to ascertain, at least for the youngest of the series, by staining with methylene blue and eosin; but in the next following stage, between which and stage 30, however, there is a small hiatus, the corpuscles contain hæmoglobin, and the megalocytes have disappeared.

The giantism of the corpuscles can hardly, however, be related to the hæmoglobin formation, because there are other hæmoglobin-free cells in the blood, very different in character. These are much smaller cells, and belong to several different categories. *First*, there are elements having all the characters of the later red corpuscles, except that they have round nuclei (figs. 8 and 9, Pl. I.), which have precisely the same characters as those of the large yolk-laden corpuscles. As all intermediate stages are observed between these corpuscles and the large ones, there is reason for believing that they are simply the large cells in which all the yolk is used up and the coarsely vacuolated protoplasm has been reduced to a fine alveolar condition. To the same category, cells with lobed nuclei like that in fig. 15, Pl. I. probably belong. On the other hand, there are, *second*, still smaller elements (fig. 12, Pl. I.), with smaller rounded nuclei. These must arise in some other way. They are found in the heart, but perhaps more frequently in the cardinal veins and in the vitelline vessels.

The appearance represented in fig. 16, Pl. I. seems to point to one mode of formation. A large yolk-laden cell has apparently divided unequally, the larger moiety remaining among the yolk cells, and forming part of the layer of smaller cells still incompletely separated from the larger yolk cells, while the smaller portion, consisting merely of a nucleus and a small zone of yolk-free protoplasm, is budded off to become a free cell.

I have also observed one instance suggesting that a multinucleated cell is being resolved into a number of elements; but as I have been unable to discover another, I cannot speak definitely regarding this possible source of these small corpuscles. That these small elements become young erythrocytes is indicated by the occurrence of such cells as figured in figs. 13 and 14, Pl. I.

The first change in the conversion is the appearance of a delicate layer of fibrillæ in the protoplasm, forming an ill-defined ring when the corpuscle is seen on the flat, and showing as the group of apparent granules in profile view (fig. 14, Pl. I.).

Third, still another type of free cell also occurs (fig. 17, Pl. I.) in which the nucleus is polymorphic. The protoplasm is exceedingly homogeneous, and shows only very indistinctly any reticular or alveolar disposition, while there is an exquisite radial sphere and large single centrosome. These cells occur very rarely. They are in general aspect the same as the elements of an earlier stage (fig. 10, Pl. I.). I have said above that these latter possibly represented the earliest mobile stage of the blood cells, or that they were the earliest representatives of the free mobile white corpuscles. I have little hesitation in naming such a corpuscle at this stage an early leucocyte, both from its characters and because it is arising from the district of the potential mesenchyme, in which, in the next phase, the leucocytes begin to appear in large numbers.

PHASE II.

As already stated, the alimentary tract up to stage 30 is represented by a solid mass of heavily yolk-laden cells, which can only be distinguished into central large and peripheral smaller cells. In no part is there any epithelial disposition of these primitive hypoblast cells. Immediately after this stage the gut begins to be cut off from the mass of yolk cells, at first as a solid cord with a palisade grouping of its cells. This cutting off of the alimentary tract is associated with the differentiation of a layer of splanchnic mesenchyme round it. I am not here concerned with the details of the process, nor with its general significance, but only with its special significance regarding the history of the blood. The only point I desire to state is that the splanchnic mesenchyme of that part of the alimentary tract from the pharynx to the region in which, later, the pancreas rudiment lies, is laid down as a direct derivative of the layer of smaller yolk-laden cells which seem simultaneously converted into the definitive hypoblast and the mesenchyme. The two layers are at first absolutely continuous, the line of demarcation being indicated only by the retention in the hypoblast for a longer period of the yolk grains. Later, however, they are sharply marked off by the rounded hypoblast elements assuming the form of epithelial cells.

Up to this point I think the evidence is fairly clear that the blood corpuscles increase in number both by direct division and also by new formation. This new formation can only be from two sources—from the somatic mesenchyme as the vessels are formed in it, or from the layer of yolk cells lying beneath the splanchnic mesepithelium. Both probably before, both almost certainly now, share in contributing to the blood; but as the definitive hypoblast and the splanchnic mesenchyme are now differentiated, a new phase is inaugurated.

I shall first enumerate the different types of free cells met with in the blood, and then refer to their seats of origin.

Four stages (—31, 31+, 32, and 32+) may be taken together for the classification of the types, though in referring to their origin I shall have to discriminate between 31 and 32. As stage 32 was the one selected for the study of the cytological characters of the corpuscles, I must refer to the plates published with Part I. of this memoir for most of the illustrations.

1st. *Erythrocytes*.

All the giant corpuscles have now disappeared from the blood. The corpuscles have assumed their definitive disc shape (figs. 1 and 2, Pl. I., Part I.). They now contain hæmoglobin. Three varieties occur, differing in the characters of the nuclei. The great majority have oval nuclei with a very coarse homogeneous chromatin reticulum, which takes the orange dye from the mixture of Ehrlich: a certain proportion have round nuclei and a relatively small cell body, while many have vesicular nuclei. As the

vesicular condition of the nucleus is characteristic of the great mass of the erythrocytes in all the later stages, we may take it that the corpuscles with this character are the mature elements. I may here remark that after stage 32 the number of dividing erythrocytes gradually diminishes, until in later phases they are very rare except in the spleen, where it is only the young erythrocytes which are dividing. I believe that the mature erythrocyte is probably incapable of division. Those with the oval nuclei showing a reticulum are therefore young corpuscles, and at the stages under consideration they are in very active division. Those with round nuclei are probably transitional forms between the erythroblast and the young erythrocyte; they are also in active division.

2nd. *Erythroblasts.*

These are small cells with a round or oval nucleus and small cell body, which, though basophile, has a delicate concentric fibrillation. Such cells are represented in figs. 18, 19, Pl. I., and fig. 24, Pl. III.). There are two stages in the fibrillation of the protoplasm. The cell drawn in fig. 24, Pl. III. has only a narrow zone of protoplasm and the fibrillation is extremely faint. In fig. 18 there is a distinct marginal band, with a zone of apparently alveolar protoplasm round the nucleus; when seen in profile section (fig. 19, Pl. I.) the band shows at the extremities of the oval body as a series of fine dots.

The characters of the nucleus are very important. Compared with the mononuclear cells of this stage and with the young red cells of the blood at stage 30, it is seen that the chromatin nucleoli are larger, massed closer together, and the intervening reticulum is coarser, so that the nucleus stains more deeply. These cells apparently correspond to what GIGLIO-TOS* calls 'thrombocytes,' after DEKHUYZEN. He regards them, not as stages of the red corpuscles, but as special elements derived from leucoblasts, and distinguished from the erythroblasts by several characters, one of which is the concentric disposition of the fibrillæ of the protoplasm, while in the erythroblasts the filaments are radially arranged. It is just the concentric fibrillation of the protoplasm, leading up to the fibrillar band, which determines in this case the nature of these cells.

3rd. *Large Mononuclear Cells.*

I have given this name to these elements because of the large simple nucleus, which is either round or notched (figs. 24, 25, Pl. III., Part I.). It is distinguished from the erythroblast by the more purely basophile reaction of the protoplasm, which is never fibrillar, showing only an extremely delicate reticulum. The nucleus has its chromatin nucleoli rather widely scattered; they are relatively small, and the intervening reticulum is very delicate and filamentous. It is to be specially noticed that all the corpuscles up to stage 30, when the hæmoglobin appears, have nuclei of this type. The mono-

* *Arch. Ital. de Biol.*, T. xxix., 1898, and *Mem. a. R. Accad. delle Sc. di Torino*, s. ii. t. xlvii.

nuclear cells vary in size, some being smaller and also showing sometimes a deepish cleft (fig. 25, Pl. III.).

The contrast between the nuclei of the mononuclear cells and the erythroblasts is important. It corresponds, in general terms, exactly with the contrast between the erythroblast and leucoblast in higher forms, as established by LOWIT and then DENYS, and more recently, still further elucidated by PAPPENHEIM.*

4th. *Small Mononuclear Cells.*

These have a small round or notched nucleus, with a relatively dense disposition of the chromatin and a narrow zone of delicate hyaline basophile protoplasm.

5th. *Leucocytes proper.*

These are found sparingly in the blood, but crowded in the spaces of the mesenchyme. Outside the blood stream they are simply free wandering mesenchyme cells. They vary greatly in size; the nucleus shows all degrees of polymorphism; there is always a large centrosome and sphere; the protoplasm is either hyaline and basophile, or granular in various degrees, from a few scattered extremely small granules to larger bodies filling the whole cytoplasm, when the cells have all the characters of eosinophile leucocytes. In a few instances the granules have a basophile reaction, taking the blue instead of the eosin from a methylene blue and eosin stain.

In the succeeding section the origin and inter-relation of these various elements will be considered.

The four stages under consideration present a gradual unfolding of the conditions which characterise the second phase in the development of the blood.

The disposition of the splanchnic mesenchyme at stage 31, may be gathered from a section, passing through the liver just behind the pharynx and heart. The solid cord of cells which represents the future stomach is seen passing down on the left of the liver to be continuous below with the still undifferentiated yolk cells. Surrounding it, and passing below over the surface of the yolk, is a very cellular tissue, which also here surrounds the solid rudiment of the bile duct, and passes along it into the liver.

This mesenchyme is a loosely arranged tissue, everywhere permeated by irregular spaces containing red blood corpuscles, but without definite endothelial walls.

The component cells (fig. 2, Pl. II.) are both free and fixed. The latter seem to be elongating to form spindle-shaped elements, while the former appear to lie free in the intercellular spaces. These free cells are already of more than one variety. The majority are large mononuclear cells, but there are also cells with metamorphosed nuclei, and some even are distinctly granular. The layer is directly continuous with the underlying hypoblast, in which mitotic figures are observed.

* *Archiv f. path. Anat.*, vol. 151, 1898. PAPPENHEIM gives a very extensive bibliography. His theses, summarised at the close of his paper, agree in respect of these blood cells closely with those of this paper.

In the vessels in the liver there are numerous cells identical with the free mesenchymatous elements, and it is to be noticed that the spaces in the mesenchyme are continuous with the venous spaces in the liver.

Turning to the somatic mesenchyme*—there is round the pronephric duct and mesonephric tubules a specially cellular tract (fig. 21, Pl. II.), which is becoming canalised, as it were, from before backwards, to form irregular venous spaces round the tubules, as I shall show immediately. The section drawn (fig. 21) is far back in the

FIG. 1.—Section through larva, stage 32+, behind heart, but in front of undifferentiated yolk cells. $\times 33$ d.

A, aorta; L, lung; Li., liver; Pr., pronephros; G, glomerulus; S, gullet; P.V., vena advehens of liver; H.V., vena revehens of liver; Y, mesenchymatous tissue covering anterior surface of mass of undifferentiated yolk cells.

series, and the tissue is not yet here penetrated by spaces containing blood corpuscles, but some of the cells seem to occupy spaces in the protoplasmic meshwork, and a few have polymorphic nuclei. The venous spaces naturally communicate with the cardinal vein, and these vessels contain cells, having again all the characters of the free elements in the mesenchyme. This tract of mesenchyme is the rudiment of the lymphoid tissue, so called, of the kidney. This has long been well known as a seat of blood formation in

* While it is quite legitimate to call the cellular tissue round the gut and on the mass of yolk cells *mesenchyme*, it is perhaps not strictly correct to use the term as applied to this tissue. I use it in quite a general sense as a convenient word to indicate the young connective tissue. Through the whole larval stages the so-called lymphoid tissue is mesenchyme in this sense, with free cells in its meshes.

the Teleosteans (BIZZAZERO, ZIEGLER, LAGUESSE, FELIX, and others). In the *Lepidosiren* larva the tissue is not true lymphoid tissue, as we understand the term in higher forms, and there are no glands. It consists simply of a tract of branched connective-tissue cells, with wide intervening spaces in which there are free lymph cells. Throughout the whole of larval life it has much the appearance of the mesenchyme at its first

FIG. 2.—Section through larva, stage 32+, further back than figure 1, through liver ducts. $\times 33$ d.

A, aorta; L, lung; Pr., pronephros; G, glomerulus; S, stomach; H.V., vena cava of liver; Y, mass of undifferentiated yolk cells; M, mesenchyme covering mass of yolk cells.

appearance, and is, as it were, a tract of that tissue which has retained its undifferentiated characters.

FELIX* shows that in Salmonidæ it is not true lymphoid tissue, and he uses the term 'pseudo-lymphoid' tissue. The tissue in the Teleosts, as described by him, differs both in appearance and development from the corresponding tract in *Lepidosiren*. To this I shall return later.

At stage 32 the general disposition of parts is indicated in the diagrams in the text. In figs. 1 and 2 the still solid gut is seen lying dorsal to the liver; further back

* *Anatomische Hefte*, Bd. 8.

it descends on the left of that organ, and, as in stage 31, the cord passes into the general mass of the yolk cells.

Round the cord of cells the mesenchyme forms a layer, differing from the general somatic mesenchyme in being composed of rounded cells closely packed together. Traced back, the layer sweeps over the yolk, surrounds the vessels and ducts entering the liver, passing with them to join the general connective tissue of that organ.

The very anterior edge of the ventral mass of yolk cells is indicated in fig. 1. As we shall see later, this tissue is composed entirely of mesenchyme, and it passes into the liver round the portal vein.

Behind the point where the definitive hypoblast of the solid gut joins the general mass of yolk cells, a mass of the mesenchyme projects free (text fig. 3) into the body

FIG. 3.—Section of larva, stage 32+, through posterior edge of liver, behind point where the solid gut rudiment becomes continuous with undifferentiated yolk cells. $\times 33d$.

A, aorta; L L, lungs; Y, mass of undifferentiated yolk cells; M, mesenchyme on surface of mass of yolk cells; N, specialised tract of mesenchyme along pronephric duct.

cavity to the left of the hinder end of the liver. On each side this fades away into the general mesendodermic layer of the yolk, while behind this point the splanchnic mesenchyme, as such, is absent (text fig. 4), until again posteriorly it surrounds the solid hind gut which is being differentiated from behind forwards (text fig. 5). The general somatic mesenchyme is at this stage formed of widely separated stellate or fusiform cells, but the tract adjoining and surrounding the pronephric duct, the pronephric funnels, and mesonephric tubules has quite special histological characters. This tract can be followed all along the body of the embryo.

Before proceeding to describe the minute characters of the splanchnic mesenchyme and of this specialised tract of the somatic mesenchyme,* it may be observed, as a fact

* See note, p. 464.

of possible significance, that this specialised tract corresponds in situation to the original seat of origin of the 'sclerotome' from the mesoblast;* while the splanchnic mesenchyme is the differentiated representative of a previously undifferentiated mass of primitive hypoblast cells which had certainly an important share in the contribution of the early blood corpuscles.

I must also state that I have searched more than once every section through the pharynx and gill clefts at this stage for thymus 'placodes' giving origin to leucocytes, as described by BEARD.† I cannot identify any thymus rudiment, nor have I seen any appearances like those figured by BEARD. Moreover, though leucocytes occur here

FIG. 4.—Section through larva, stage 32+, behind liver. $\times 33$ d.
A, aorta; C.V., cardinal vein; N, specialised tract of mesenchyme in region of meso-*ephras*.

and there in the general mesenchyme in that region, and also in the aortic arches, I do not happen to have seen a single one in the anterior cardinal vein.

Splanchnic Mesenchyme.

It has already been observed that this tissue surrounding the isolated portion of the gut is composed of rounded closely packed cells. There are numerous spaces in it containing red blood corpuscles, and here and there free elements with characters similar to those of the cells immediately to be described.

Dorsal to the gut, a tract is showing the first stages of differentiation from behind forwards, which will convert it into the spleen. The layer on the yolk which is in direct continuity with the undifferentiated hypoblast has, however, very special characters. If a section be taken at Y in text fig. 1 it will be seen that the tissue is composed of loosely arranged fusiform or stellate cells, with many free elements in the

* I find a remark almost in the same terms as this was made by ZEIGLER (*Arch. mikr. Anat.*, xxx., 1887), referring to the similar tract in *Teleosts*. See also LAGUESSE, *loc. cit.*, p. 364.

† *Loc. cit.*

spaces between them. These are either of the mononuclear type or, more frequently, show various degrees of polymorphism, and a large number have their protoplasm filled with granules. Further back, the tissue over the surface of the yolk has the same characters, and in certain situations, as at the point marked M in text fig. 2, it has the appearance represented in fig. 22, Pl. II. Above is the line of the mesepithelium, below and to the left is the yolk-laden hypoblast. Directly continuous with this the mesenchymatous tissue is composed of a network of protoplasmic threads, in the meshes of which are cells showing all degrees of polymorphism in their nuclei, while in the deeper layers near the hypoblast there are numerous cells with the protoplasm laden

FIG. 5.—Section through larva, stage 32+, at level of the hind-limb buds.

A, aorta; C.V., cardinal vein; Pr., nephric duct between Pr. and A, the tract of mesenchyme lettered N in preceding figures; G, solid hind gut.

with fine granules. The nuclei of the cells forming the framework are widely separated, and are thus apparently fewer in number than the free cells. They are elongated like the nucleus above and to the left (bounding here a space containing two erythrocytes), or rounded like those below, between the two horseshoe-shaped nuclei.

In other portions of the tissue the general characters are the same, but the polymorphic metamorphosis of the nuclei is not everywhere so pronounced, and large mononuclear free elements are more numerous. Throughout this tract of mesenchyme mitotic figures are of frequent occurrence.

Somatic Mesenchyme.

In the tract above mentioned the appearances differ according to the degree to which the tissue round the nephric ducts is opened out into venous spaces communicating with the cardinal vein.

In the region of the pronephros (fig. 25, Pl. III.) the duct is surrounded by an irregular sinus, interrupted here and there by delicate protoplasmic strands, belonging to cells of an endothelial character, with elongated nuclei. The sinus is crowded with dividing erythrocytes and with large mononuclear cells, with round, pitted, or notched nuclei. Here and there, there is a free polymorphonuclear corpuscle, either with hyaline protoplasm or with various degrees of granulation. Within the spaces of the trabecular framework are many polymorphonuclear cells, in some cases having all the characters of eosinophile leucocytes.

Further back (fig. 23, Pl. II.), in the angle between the cardinal vein, aorta, and duct (text fig. 5), the tissue is not so much opened out, the framework is closer, and in its meshes are massed free elements with round, notched, or polymorphic nuclei and various degrees of granulation. In the actual blood spaces the great majority of the cells are of the large mononuclear variety.

In the region of the mesonephros the general characters of the tissue round the tubules is similar, as will be gathered at once by reference to fig. 26, Pl. III.

The individual free cells outside the blood stream vary greatly in size, without any further indication of discriminating characters. Mitoses are frequent, but there is nothing to indicate that one type of cells is dividing to give origin to another. They seem to divide at various stages in the metamorphosis of the nucleus, and all after their kind. In fig. 24, Pl. III. there is drawn a cell in one of the venous spaces, which from its more crowded nuclear reticulum and the indication of a faint fibrillation of its protoplasm is to be regarded as an erythroblast, while outside the blood stream is a mononuclear cell of the largest variety, which in general dimensions is equal to the erythroblast.

The cardinal vein, compared with the aorta, contains a disproportionate number of white to red elements, and a preponderating number of mononuclear cells.

Thus in 112 sections the white elements were distributed between the two vessels thus:—

	Small Mononuclear.		Large Mononuclear.		Polymorphonuclear.
Aorta,	8	...	8	...	6
Cardinal vein,	14	...	100	...	26

The cardinal vein, however, being much larger than the aorta, it was necessary to arrive at the proportion of the white to the red elements in each. This was arrived at

by counting the erythrocytes and white cells in every third of the 112 sections. The figures were as follows :—

	Erythrocytes.		White Cells.
Aorta,	1003	...	22
Cardinal vein,	2634	...	140

The proportion of red cells in the aorta compared with the cardinal vein was thus less than 1 to 3, while the proportion of white cells was nearly 1 to 7.

I have used the term 'white cells' rather than 'leucocytes' here because the counts include the large mononuclear cells, which are not necessarily all true leucocytes. Those in the spaces of the mesenchyme outside the blood sinuses are certainly in great part 'leucoblasts,' in the sense that all the polymorphic and granular cells are derived from them by metamorphosis of the nucleus and by the deposition of granules in their protoplasm; but what is the relation of those within the blood stream to the non-hæmoglobin-containing erythroblasts?

As one has, unfortunately, no opportunity of actually seeing one form of cell change into another, this question can only be answered in terms of probability.

A careful scrutiny of these intravascular mononuclear cells in the cardinal vein and the spaces communicating with them shows that certain of them have rounder and larger nuclei than others, though identical in general characters.

Several considerations point to the probability that these cells are progenitors of the erythroblasts.

1st. The polymorphism of the erythrocyte series is in favour of the view that the blood is at this stage receiving new formed elements. There seems no reason why certain corpuscles in the general blood stream should retain, under the same conditions, their primitive generalised characters if all the corpuscles are survivors from earlier stages.

2nd. If new elements are being added, the fact that erythroblasts are never found outside the blood stream indicates that they must be derived from less specialised cells in the blood stream, which in turn should have the characters of cells outside the blood stream. The large mononuclear cells fulfil the conditions and complete a logical chain.

But the question arises as to the relations of the cells without and within the blood stream. Numerous cases prove that cells are passing from the spaces in the mesenchyme into the blood stream, or *vice versa*, but it is not possible absolutely to say in which direction they are moving.

The fact that these mononuclear cells occur in disproportionately large numbers in a vessel,* the current in which would carry them away from this locality, indicates that in all probability they do arise in this tract of mesenchyme, and are there set free

* The sections in which the above count was made were behind the liver, a long distance posterior to the point where the hepatic vein joins the cardinal.

in the blood stream. From the evidence available, it seems to me justifiable to conclude for this stage---

1st. That new elements are being added to the blood stream. These belong either to the erythrocyte or leucocyte series.

2nd. In regard to the leucocyte series, all the cells free in the blood which can be called leucocytes are derivatives of the mesenchyme; certainly of the splanchnic, almost certainly of the nephric tract of somatic mesenchyme.

3rd. In regard to the erythrocyte series—the erythroblast without hæmoglobin is derived from an intravascular ‘mononuclear element,’ which is in turn derived from the mesenchyme, probably from both splanchnic and somatic mesenchyme.

4th. The two series arise from a common mother cell—the mesenchyme cell. The changes which convert the mother cell into an erythrocyte take place in the blood stream, while its metamorphosis into a leucocyte is brought about *in situ*, in the spaces of the mesenchyme outside the blood stream.

Before leaving this stage I must refer to the character of the granulation in the granular leucocytes. In some instances, as I have already stated, these cells have all the characters of eosinophile cells. It would take me out of my direct way to go into the general question of the meaning of the granulation of leucocytes; and I would here merely remark, in regard to these particular cells, that the granulation may be merely yolk material in fine division, for I have observed precisely similar eosinophile granules in the yolk cells, and the yolk itself has strong affinity for eosin. At this stage I find the granular cells crowded together only in the splanchnic mesenchyme and in the tract of the somatic mesenchyme so frequently alluded to. It at once suggests itself, in the case of the cells near the yolk, that this may be the source of the material which constitutes the granulation; and in regard to those in the neighbourhood of the nephric duct, it is worth mentioning that the cells forming the wall of that duct are filled with yolk grains long after they have disappeared in the neighbouring tissues. It seems to me not impossible that these early leucocytes may be concerned in the distribution of yolk food. Were this the case, the granules would necessarily differ from those of the leucocytes of later stages, but I see no inherent improbability in the suggestion, which, if well founded, has a bearing on the nature of granulation in leucocytes generally.

PHASE III.

After the second phase, as I have ventured to define it, is fully established at stage 32 or 33, the general conditions are maintained for a time, during which the histogenesis of the spleen is gradually accomplished and the atrophy of the pronephros completed. It is with the complete differentiation of the spleen as a hæmopoietic organ that what I call the 3rd phase in the history of the blood is established, and the renewal of the blood corpuscles is confined to that organ, and to the pseudo-lymphoid tissue round the kidneys, and possibly also round the gut. It is presumably the adult state of things

that is thus established. At no stage does the liver take any part in blood formation. The part taken by the spleen and the lymphoid tissue of the mesonephros and gut in the process will now be considered.

Histogenesis of the Spleen.

As already mentioned, the rudiment of the spleen appears in the splanchnic mesenchyme dorsal to the gut. It is simply a differentiated tract of that tissue. The hypoblast takes no direct share in its formation, but it must be observed that it takes form in the mesenchyme very shortly after that tissue is itself differentiated from the primitive undifferentiated mass of yolk cells.

The first sign of its formation is the appearance of a column of large rounded or oval cells, round which the remaining cells of the mesenchyme group themselves concentrically (fig. 28, Pl. IV.). The nuclei of the investing cells are oval or elongated, and their long axes are arranged in a general way parallel to one another, and concentrically to the central tract. As elsewhere, this tract of mesenchyme is permeated with spaces having no definite endothelial walls, but containing red blood corpuscles.

These spaces are at first irregular, but in the second stage (fig. 29, Pl. IV.) they run together so as to form a peripheral sinus surrounding the central tract, or island as it appears in sections, isolating it in great measure from the peripheral layers of investing cells. These latter become the investing connective-tissue coat of the spleen. The central tract is permeated by cleft-like spaces which communicate with the peripheral sinus. Both spaces and sinus contain red cells, and in the sinus are seen a number of leucocytes, evidently wandering in from the general mesenchyme. In the central mass, here and there are seen cells with simple nuclei surrounded by a layer of free protoplasm. Numerous mitotic figures occur among the cells of the central tract, which by multiplication of its constituent cells increases in size, while at the same time its spaces are opened out until (fig. 30, Pl. IV.) it is converted into a system of cellular columns or trabeculæ. The peripheral sinus is now less definite in its arrangement, because the spaces between the outer ends of the columns have enlarged and become continuous with the lumen of the sinus. The peripheral investing cells are now all flattened connective-tissue elements, and the capsule of the organ is complete.

The sinuses at this stage are full of red cells, nearly all of the mature variety. There are few erythroblasts, the phase contrasting in this respect markedly with the next, in which the sinuses are filled with young erythroblasts.

The cellular columns are composed of cells of various dimensions (fig. 3, Pl. IV.). Some have smaller and irregular nuclei, but the great majority have round or oval nuclei of large size. In the spaces within the trabeculæ there are numerous white elements (fig. 31, Pl. IV.). These have either simple nuclei and hyaline protoplasm, or polymorphic nuclei and hyaline or granular protoplasm. The polymorphic nuclei have a closer arrangement of the chromatin nucleoli than the cells with the simple

nuclei, so that they take a deeper coloration. The shape of the nucleus varies, but is most frequently horseshoe-shaped, and the protoplasm is either hyaline or granular in different degrees. Though it is true that at the earlier stages leucocytes are wandering in from without, I think there is little doubt that these cells are being formed at the expense of the cells of the columns or trabeculæ, which, after all, have exactly the same origin as the remainder of the mesenchyme, in which we have seen the cells being transformed into similar elements.

The mononuclear cells have certainly a local origin. They are seen crowding the splenic vein (fig. 27, Pl. III.). They are of varying dimensions, and are indistinguishable from the cells with simple nuclei first observed in the blood at stages 31 and 32. The problems regarding them are exactly the same as presented themselves at the earlier stages, but I shall postpone discussion of the point until I consider the next stage, as I have had the opportunity of staining a series of sections freshly cut from that stage with stains specially suited for discriminating between the different types of cells.

At stage 37 the spleen is an elongated organ, overlapping the pancreas behind and extending forwards along the whole length of the liver to a level close behind the point where the lung comes off from the pharynx. It is broad behind, but tapers in front, and it is in this anterior part that the general structure can be most easily determined (fig. 32, Pl. IV.).

Surrounding the central artery there is an axial mass of cells with small irregular nuclei. From this to the periphery extend radiating trabeculæ, with small oval or elongated nuclei. Between the trabeculæ are large sinuses packed with cells belonging to different categories, and in the meshes of the framework immediately round the central area are large and round nuclei, which sometimes, it is quite clear, have a zone of free protoplasm round them, while in other cases they seem imbedded in the general protoplasmic framework. In the apex of the organ the structure is simple because this zone is much reduced, but posteriorly it is more extensive and more loosely arranged, so that the picture is more complicated. It is a matter of great difficulty to say sometimes whether the large round nuclei with which this zone is studded belong to the trabecular framework or to cells in its meshes.

In the peripheral sinus, and the larger sinuses between the outer ends of the trabeculæ which are continuous with it, there are great numbers of hæmoglobin-containing erythroblasts. These and the erythrocytes give to the outer zone of the spleen characters which would justify the application to it of the name of 'pulpe rouge' used by LAGUESSE,* while the central portion is the 'pulpe blanche.'

The free cells in the spleen pulp belong to several different categories, and the discrimination between them is a matter of difficulty, as all the nuclei stain blue with hæmatoxylin, and the only differences are those of intensity of staining, associated with a difference in the disposition of the chromatin. I was unable, except in a

* *Jour. de l'Anatomie et de la Physiologie*, T. xxvi., 1890.

general way, to identify the elements in a series stained with this dye and counter-stained with eosin.

On a new series I tried many stains, but owing to some factor in the fixative, presumably the acid, I found the tissue would not stain with any basic dye. I had recourse, therefore, to Mann's double acid mixture of methyl blue and eosin, but found that for this particular material I got much more vivid differentiation by staining with the two dyes successively, first for three minutes in a saturated watery solution of eosin, and then, after rinsing in water, in a saturated watery solution of methyl blue for one or two minutes. The results varied according to the proportion in which the two dyes were held; and although there was some variation in the colourisation, the conclusions were not vitiated, because the picture was always a relative one.

The general effect depended partly on a difference in the reaction of the protoplasm, but chiefly on the relative proportions in the nuclei of bodies with different affinities to the two dyes.

In my most successful stainings I obtained the following results:—

A. *Fixed Cells.*

The protoplasmic trabeculæ stain pure blue, and the nuclei have a general blue tint owing to the general reticulum of the nucleus selecting the blue dye, but the nucleoli (chromatin) stain a violet-red colour. The central parts of the karyosomes stain yellowish-red. The violet colour is given by an outer covering or coating, as it were, of the blue staining general linin reticulum.

B. *Free Cells.*

1. In the meshes of the trabeculæ there are large numbers of cells with a large nucleus and a small amount of blue-staining protoplasm. The nuclei are round and vary in size, but roughly they may be divided into two classes—those with a diameter about 24 μ and those with a diameter of about 18 μ . Many have smaller nuclei, but as there are frequent mitoses they may be considered young cells. The nuclei are characterised (fig. 33, Pl. IV.) by the large amount of blue they select, the general fine reticulum taking up the methyl blue, while the chromatin nucleoli, which are relatively few in number, stain reddish-violet. The characters are thus exactly the same as the nuclei of the reticulum.

2. Distinguished from these cells are others which have nuclei of the same dimensions as the largest of the cells of the last category, but which react differently to the stain (fig. 33, Pl. IV.), their general tint being reddish-violet. This is due to a difference in the disposition of the chromatin. The nucleoli are larger and are more closely opposed, and the blue-staining reticulum between them is reduced, and has a violet, not a pure blue tint. These cells are very frequently seen dividing, and are distinguished during division by the red-violet colour of their chromosomes. The daughter cells have

naturally smaller nuclei, but are distinguished by the reaction of the nuclei. The protoplasm has an open reticulum, but is relatively small in amount. The outer layers are frequently seen to be fibrillar, the fibrillæ being arranged circumferentially. There are also fine reddish granules in many of the cells, which, I take it, would correspond to GIGLIO-TOS's hæmoglobigenic granules.

3. The next class of cells is found only in the large sinuses. The nucleus is of the same dimensions as that of the cell just described; it is round or oval, and selects only the eosin; the colourisation is reddish-yellow, the nucleoli are closely packed, and joined into a coarse reticulum by bars staining yellow like the rounded masses of the nucleoli. The mitotic figures, which are very numerous, belonging to this class of cell, are distinguished by their yellow chromosomes. The protoplasm varies in amount; it has an indefinite warm tint, and many of the cells have yellow granules. The peripheral layer is fibrillar, and round the nucleus the protoplasm shows a wide-meshed reticulum.

Looking at these three varieties of cell, it is clear that the third is an erythroblast containing hæmoglobin. It is almost equally clear that the second is a stage of the third—that, in fact, it is a primary erythroblast. The spleen, therefore, is a seat of origin of the erythroblasts, but do they arise by multiplication of erythroblasts which have entered the organ from without, or by new formation from the spleen cells? I conclude for the latter alternative, for the following reasons.

While it is possible that the erythroblasts having nuclei characteristic of hæmoglobin-containing corpuscles might be derived by division from the red blood corpuscles in the sinuses of the previous stage, it is not possible that cells with nuclei which do not show that reaction should be derived from hæmoglobin-containing corpuscles. They might arise, however, by division from non-hæmoglobin-containing erythroblasts derived from without, but their numbers are far out of proportion to the number of mitotic figures, so that one is driven to believe them to be a further phase of the larger cells of the first category. As a matter of fact, in cells with all the characters of these, here and there one occurs with a definite concentric fibrillar condition of the protoplasm (fig. 33, Pl. IV.), which I take to be the first stage in the conversion of the cell into an erythroblast.

I think, again, that it is reasonable to derive these cells of the first category from the mesenchyme of the original rudiment. It is very hard to say at this stage whether their protoplasm is actually free, or part of the general protoplasmic framework; and as this is the last stage of the series available, I am unable to say whether any part of the original cellular columns is retained in its primitive form, to give rise continuously to new budded-off elements; or whether the cells are all set free in the meshes of the reticulum, and give rise by continuous division to new elements.

It seems justifiable to conclude that the original spleen cells, by a series of changes in the protoplasm and nucleus, become converted first into non-hæmoglobin-containing erythroblasts, and that these, in turn, acquire hæmoglobin and become the young red cells, and that the cells I have described represent the stages in the process.

Besides the erythrocyte series, there is a large leucocyte contingent in the spleen. The fully unfolded leucocytes are of two main classes. *1st*, Small cells, with horse-shoe or lobed nuclei, with rather closely packed chromatin nucleoli, and a small cell body composed of hyaline blue-staining protoplasm, though sometimes it contains granules. *2nd*, Large cells, with polymorphic nuclei and a large cell body, in which the centrosome is always surrounded by a well-developed radial sphere and aster. The protoplasm is either hyaline and blue-staining or granular, and the granulation is either fine or coarse. The fine granules stain in some cells blue, in others red, and the coarse granulation of the eosinophile cells is not always of the same size.

Compared to the erythroblasts, the leucocytes are in relatively small numbers, but in the spaces and sinuses there are many of the cells which have all the characteristics of leucoblasts (fig. 33, Pl. IV.). The nuclei vary in size, but generally speaking are smaller than those of the largest spleen cells ($18\ \mu$ against about $24\ \mu$), but have otherwise the same character and reaction.

The protoplasm is reduced to a very narrow zone, is hyaline, and stains pure blue. Many nuclei are deeply notched (fig. 33, Pl. IV.), as if beginning to undergo polymorphic metamorphosis.

As leucocytes in all varieties are abundant in every tissue of the body, and especially round the kidney tubules and gut. as I shall presently describe, it is much less certain whether they are actually formed in the spleen at this stage than that the erythroblasts are rising there.

The great difficulty is, that it is impossible to distinguish a leucoblast from a primitive spleen cell. We have here repeated the same problem dealt with before in connection with the origin of the two classes of corpuscles from the mesenchyme cells; and considering that the spleen is merely a tract of mesenchyme, which may be supposed to retain its primitive potential characters, the same general scheme may not unreasonably be considered to apply, which would derive from the primitive cells of the rudiment both classes of corpuscles by specialisation along different lines. My observations on the spleen are thus in strict accord with those of LAGUESSE* in his classical work. My large mononuclear cell takes the place of his *noyau d'origine*, a term borrowed from POUCHET,† who first formulated the general scheme here adopted from his work on the blood of Triton.

The leucocytes, in their several varieties, are found in the blood-vessels and scattered in every tissue, but are specially crowded in the wall of the gut in the mesentery and in the tissue surrounding the tubules of the kidney.

Pseudo-lymphoid Tissue of Kidney.

The tract described at stage 32 has disappeared in front with the atrophy of the pronephros, but it can be identified in the region of the kidney, and the general

* *Loc. cit.*

† *Gaz. Méd. de Paris*, 1879.

appearances are much like those figured at stage 32 in the region of the mesonephros (fig. 26, Pl. III.).

The tubules are imbedded in what is practically a huge sinus; in the blood stream a great variety of elements occur. There are numerous erythroblasts, many mononuclear cells, and numbers of leucocytes, while in the pseudo-lymphoid tissue there are large quantities of leucocytes of various kinds, but the polymorphic and granular varieties preponderate. The general impression when a section is compared with a section of the spleen is, that while in the latter the primary erythroblasts and secondary erythroblasts are greatly in the majority, the reverse is the case in the kidney.

I have made some attempts to arrive at some estimation of the relative numbers of erythroblasts and leucocytes in the renal-portal and cardinal veins. As the vessels are, in the greater part of their course, unequal in size, any count, to be a reliable index of the part taken by this tissue round the kidney, in contributing new elements to the blood, would require to be one relative to the number of erythrocytes. This I found impracticable, on account of the corpuscles being unequally distributed. I therefore, to reduce the balance in a rough way, counted in every fourth section of a continuous series of 200 the erythroblasts and leucocytes in one renal-portal, and put them against those in the two cardinals. I have not sufficient confidence either in the method or the figures themselves to submit them in detail, or to found a definite judgment on them, but I may say that the general result was in favour of the renal-portal as regards both erythroblasts, large mononuclears, and leucocytes; and that while erythroblasts and large mononuclears of the type seen issuing from the spleen pulp were very common in the renal-portal along the whole length of the kidney, they were practically absent in the cardinal. The possible explanation is, that in the 'backwater' formed by the great kidney venous sinus, the erythroblasts and their mother cells undergo their further transformation into erythrocytes.

Thus, though I have shown that it is highly probable that both orders of corpuscles are produced along this tract at an earlier stage, it seems doubtful whether in the later phases the pseudo-lymphoid tissue of the kidney is concerned in the new formation of red cells. I have shown how the spleen, at first distinctly lymphoid, becomes later more specially concerned in the formation of the erythrocytes, and there is some reason for believing that the kidney tract is differentiated in the opposite sense.

The development of this pseudo-lymphoid tissue, according to my account, is very simple. It is nothing more than the mesenchymatous tissue round the nephric duct and tubules, canalised, as it were, by venous spaces which communicate with the cardinal vein. From the first the cells are fixed and free; the fixed cells form the general connective-tissue basis; the free cells are either derived from the primitive mesenchyme cells of the tract itself, maintained by constant division, and set free in the blood stream by their own amœboid movement, or they wander to this site from the splanchnic mesenchyme. I have shown reasons for a belief in the first alternative.

This account is different from that given by FELIX* of the development of the corresponding tissue in the Salmonidæ. He derives the tissue from proliferation from the wall of the vein, and he describes and figures trabeculæ of *epithelioid* cells which give rise to the tissue, the cells in places becoming converted into both white and red elements. I have not seen appearances like these in *Lepidosiren*. In the early stages there is no sharp separation of the tract laden with lymphoid cells from the general mass of the mesenchyme, and all the indications are in favour of the view that it is merely a tract of the general mesenchyme in the spaces of which, for physiological reasons, the lymph cells are congregated. §

This lymphoid tissue in the larval kidney is the rudiment of a very remarkable mass of lymphoid tissue in the cortical part of the adult kidney. It forms a thick cap, so densely filled with pigment that the structure is quite concealed, but it can be seen that it is thickly studded with leucocytes. †

Lymphoid Tissue in Gut Wall.

The fact that GIGLIO-TOS ‡ describes the spiral valve as the hæmopoietic organ in the lamprey directed my attention specially to that structure in the later larval stages. I find that the fold is occupied by a denser tissue than that investing the gut. The small elongated nuclei proper to the tissue are more closely packed, while the free lymphoid cells or leucocytes are much less numerous.

The tissue of the spiral valve does not then differ in kind from the general investing tissue of the gut wall, and so far as the larva is concerned there are no appearances suggesting that it has a special hæmopoietic function.

The number of leucocytes in the connective tissue investing the gut is very great. They lie in the meshes of a loose alveolar reticulum, and belong to all the different categories. There are large numbers of mononuclear cells, here in every probability, leucoblasts. Mitotic figures occur frequently. Cells with all degrees in the metamorphosis of the nucleus, and with granules of all the varieties already mentioned, are very plentiful. Just as in the case of the kidney, this tissue represents, doubtless, the early stage of the lymphoid tissue surrounding the gut and occupying the spiral valve of the adult animal. This tissue is probably purely lymphoid—or, rather, the free cells are probably all lymphoid elements. There are no vessels to be seen crowded with erythroblasts, such as one would expect to find if the tissue had any relation to the formation of red cells.

Before closing the descriptive part of my paper, I must refer to the condition of the thymus gland at this stage. I have not studied the early stages of its development, but I now find it as a small organ, cut up into lobes by the passage through it of muscular fibres and a nerve cord. The cells of the gland are now free from the

* *Loc. cit.*

† See paper by GRAHAM KERR, *Proc. Zool. Soc.*, 1901–2.

‡ *Arch. Ital. de Biologie*, vol. xxvii., 1897.

§ Cf. ZIEGLER, *loc. cit.*, 1892, note, p. 21.

hypoblast, and though differing from the cells lining the gill clefts and pharynx, in respect of their limited protoplasmic envelope they have a definitely epithelioid appearance. The nuclei are closely packed, are round or oval, and in no single instance fissured, horseshoe-shaped or lobed. In the whole gland I have detected only one leucocyte—an eosinophile, and therefore a mature variety. The appearance of the cells is wholly different from that of any class of the leucocytes; and while these elements occur in their thousands round the gut in the spleen and kidneys, and scattered in every tissue, only one or two occur in the neighbourhood of the gland.

The so-called lymphoid transformation of the epithelial cells described in various forms by KÖLLIKER, PRENANT, MAURER, SCHULTZE, NUSSBAUM and PRYMAK, and BEARD cannot, so far, have taken place. Up to this stage, then, the thymus can have no share in contributing leucocytes to the blood, unless on the impossible assumption that the epithelial cells have become lymphoid, gone off as leucocytes, and become again replaced by epithelial cells.

I hold this observation to be ample proof that the leucocytes in *Lepidosiren* do not originate in the thymus in the larval stages, but I have in preparation a note on the Thymus which will bring the matter to a perfectly conclusive issue.

GENERAL REMARKS.

The divergence of opinion as to the first origin of the blood is so great that it is difficult to reconcile the various accounts. My results are suggestive in this connection, for they show how both primitive hypoblast and mesenchyme may, under certain conditions of development, share in blood formation. As in the later phases there is, in the nature of things, a degree of uncertainty, I shall attempt to summarise my facts in the strictest terms of accuracy, and then draw together such conclusions as I think they may reasonably bear.

SUMMARY OF FACT.

(1) The primary corpuscles, the first origin of which I have not studied in detail, are all alike in characters.

(2) At a stage sometime before there is any suspicion of hæmoglobin being present, there are two classes of corpuscles, one with a distinct circumferential equatorial fibrillation of the superficial layers of the protoplasm and without attraction sphere, the other without such modification of the protoplasm, but with distinct centrosome and sphere.

(3) In all later stages these two kinds of corpuscles coexist, but at stage 32 there is a sudden great increase in the proportion of the active type of cell.

(4) At stage 30, which is critical, there are (*a*) corpuscles presenting the features of intermediate stages between the large ringed yolk-laden bodies and an oval, disc-shaped,

ringed yolk-free corpuscle with a round nucleus; (b) small cells with a small amount of protoplasm formed (?) by budding (unequal division) of larger yolk-laden cells, and (c) similar cells with slight concentric fibrillation of the protoplasm.

(5) In all stages up to 30 the nucleus in every variety of corpuscle is identical except in size. In the arrangement of the chromatin it agrees with that of the large mononuclear basophile elements of all later phases.

(6) The cells from which the smaller elements are derived are located *certainly* in the mass of yolk cells under the splanchnopleuric mesepithelium, *possibly* in the somatic mesenchyme in the neighbourhood of the developing cardinal veins.

(7) From stage 30 onwards the active cells appear in progressively greater numbers up to stage 32+, in which they are very plentiful.

(8) From stage 30 onwards there are always cells with nuclei of the erythroblast type, and fine concentric fibrillation of a basophile protoplasm. Side by side with these are hyaline cells with small amount of basophile protoplasm and simple nuclei of the leucocyte type, identical with the nuclei of the earlier young red cells.

(9) The mass of yolk cells immediately under the splanchnopleuric mesepithelium begins at stage 30 to become differentiated with the growth of the liver, and isolation of the gut rudiment, into definitive hypoblast and splanchnic mesenchyme.

(10) At stage 31 the splanchnic mesenchyme is a cellular tissue with spaces containing blood corpuscles and free elements of two kinds: (a) cells identical with the large mononuclear cells appearing in increasing numbers in the blood; and (b) polymorphonuclear cells, with either hyaline or granular protoplasm. The nephric tract of somatic mesenchyme also contains a few free mononuclear and polymorphonuclear cells, but in smaller numbers than occur in the very cellular splanchnic mesenchyme. Mitoses are frequent in both tissues.

(11) At stages 32 and 32+ the splanchnic mesenchyme holds in its spaces numbers of mononuclear basophile cells, and in parts is composed almost wholly of polymorphonuclear cells, with hyaline or granular protoplasm. The whole perinephritic tissue has, massed in the spaces between its stellate cells, very large numbers of mononuclear basophile cells, with nuclei becoming transformed into every grade of lobing, and their protoplasm acquiring every degree of granulation. The portal and hepatic veins, and the cardinal vein and spaces communicating with it, contain white cells of different categories, the mononuclear preponderating. The cardinal vein contains three times as many erythrocytes as the aorta, but seven times as many white elements. The mononuclear outnumber the polymorphonuclear by four to one in the cardinal vein, while in the aorta they occur practically in equal proportions.

(12) The splanchnic mesenchyme retains its primitive characters in part round the gut along its whole length, forming the so-called lymphoid tissue of the adult.

(13) The nephric tract of the somatic mesenchyme also retains its primitive characters and forms the so-called lymphoid tissue of the adult kidney.

(14) The spleen rudiment is a tract of, at first identical, closely packed mesenchyme

cells. The outer cells, later, form the capsule; the central cells give rise to cellular trabecular tissue, which in part becomes the connective tissue of the fully formed organ, in part gives rise to free cells.

(15) In the earlier stages the splenic vein is full of mononuclear cells, with nuclei of the leucocyte type, identical with those in the blood at stage 32, before the spleen has differentiated. There are also in the vein a few polymorphonuclear cells, and these are in large numbers in the spaces of the trabeculae.

(16) In later stages the spleen pulp contains *first* basophile cells, with the leucocyte type of nucleus. In some parts the remains of the basophile cellular trabeculae are seen containing nuclei of the same type; *second*, similar cells with concentric fibrillation of the basophile protoplasm; *third*, cells with nuclei of the erythroblast type and concentric fibrillae in the protoplasm; *fourth*, cells with nuclei of the erythrocyte type, and yellow granules in the ringed cell-body; *fifth*, mature erythrocytes; *sixth*, mononuclear leucocytes with basophile protoplasm; *seventh*, polymorphonuclear leucocytes, small and large, in all their varieties. The members of the erythrocyte series seem greatly to outnumber those of the leucocyte series in the spleen.

(17) The leucocytes are in great abundance before the thymus rudiment appears, and at the end of the larval series examined, the gland is still a mass of epithelial cells, showing no resemblance to the lymphoid cells in other tissues. There are practically no leucocytes in its substance, and no special grouping of them, either in the surrounding mesenchyme or in the veins.

SUMMARY OF INTERPRETATION.

The study of the early corpuscles shows that they are at first all alike—probably wandering cells, such as those first described in the living Teleost embryo by WENCKEBACH.* Almost immediately a change sets in, which leads to the adoption by most of them of a passive rôle, while others remain free mobile elements, with centrosome and sphere.

The latter have all the morphological characters of leucocytes, and exactly similar cells occur in the somatic mesenchyme. As this is long before hæmoglobin is developed, the cells are never at any stage wholly hæmoglobin-containing corpuscles; and if these leucocyte-like cells are the successors of the early corpuscles, mobile elements are never absent from the blood. It is possible that we should call the early corpuscles themselves 'leucocytes.' This may seem an unwarrantable use of the term, but I believe it might bear examination. The leucocyte is generally admitted to be the phylogenetically older cell. In the Dipnoi the blood is extraordinarily rich in leucocytes, and they appear at a very early stage. The postponement of the appearance of the leucocytes in ontogeny, supposed to exist in all vertebrates, has, it might be considered, only

* *Journal of Anat. and Phys.*, vol. xix., 1885; *Archiv f. Mikr. Anat.*, Bd. 28, 1886.

partially taken place in *Lepidosiren*. PARKER* says that in *Protopterus* the leucocytes bear a larger proportion to the red cells than in any other vertebrate, except in pathological conditions. It is a possibility that in *Lepidosiren* we have a stage in which the blood vascular and the lymph vascular systems are not sharply marked off from one another. Perhaps we see in the blood the division of labour going on, which makes one primitive corpuscle a respiratory, another a lymph cell. The phenomena of phase 2 support such a supposition.

Up to stage 30, when the hæmoglobin appears and phase 2 sets in, all the corpuscles, active or passive, have nuclei of the leucocyte type; the chromatin is collected into karyosomes, rather widely scattered, with a fine filamentous intervening reticulum. The erythroblasts in the following stages have the erythrocyte type of nucleus, richer in chromatin, with large closely-set chromatin nucleoli, joined by a coarser and more distinctly reticular intervening substance. (Compare figs. 13, 14, 15, 19, Pl. I.) At stage 31, and better marked at stage 32, the place of the earlier cells with nuclei of the leucocyte type is taken by the large mononuclears. These have either a single or a double origin. It may be that they are all derived from the splanchnic mesenchyme, and that they wander from thence, especially into the nephric tract of the somatic mesenchyme. The distribution of cells in the cardinal veins is, however, against this, and I am disposed to believe that they arise in both situations. The point is not very material—they are mesenchymatous in origin in either case.†

Exactly similar cells to those mononuclears in the blood stream become outside the blood stream leucocytes of the several varieties. From the larger number of those mononuclears in the blood, compared to the number of polymorphonuclear cells, while the opposite proportion prevails outside the blood stream, and from their identical characters with the basophile cells of earlier stages, I believe it is a logical induction, not an assumption, that they become converted in the blood into the hæmoglobin-containing elements. Mitoses are frequent in those cells outside the blood stream, but they do not occur in the blood stream itself. This inference is confirmed by the observations on the spleen; and thus I conclude that from common mother cells added to the blood during phase 2 from the mesenchyme, and in phase 3 especially from the spleen (a derivative of the mesenchyme), are derived two families of cells, one undergoing their metamorphosis in the blood to form the respiratory erythrocytes, the other undergoing their metamorphosis outside the blood stream in the connective tissue, *i.e.* lymph spaces, to form the typical wandering polymorphonuclear leucocytes.‡

* *Trans. Roy. Irish Academy*, vol. xxx. part iii. p. 168.

† I am here assuming that the facts justify me in concluding for a continuous new formation, at least up to the end of phase 2. The statement in the text is perhaps too sweeping. If it were possible to establish that all the colourless cells were derived from the splanchnic mesenchyme, and that they wandered thence, it would be a fact of great significance, as it is derived directly from the primitive hypoblast, and it might be held that the free cells in it were directly derived from that layer. I think this would be a strained interpretation, and difficult to reconcile with the facts described regarding the nephric tract of the somatic mesenchyme, but it is a possibility which must not be overlooked.

‡ An objection might here be raised that the nuclei of the polymorphic cells may regain their simple form in the blood, but as there are many polymorphs in the blood this objection would not have much force.

This may be expressed in the following scheme :—

As I have already said, LAGUESSE adopts a scheme very closely similar to this, and the origin of both red and white corpuscles from a common mother cell in the blood was first suggested by POUCHET in 1879. The same sort of scheme was set forth by GIGLIO-TOS in his account of hæmatogenesis in the adult lamprey. My large mononuclear mesenchyme cell would also represent the embryonic form of the parent white cell postulated by PAPPENHEIM as the forerunner of the erythroblast, while from an embryological point of view my results in a general way agree with those obtained by SAXER, according to whom the red cells in the mammalia are derived from polymorphic mesenchyme cells, while the leucocytes are derivatives of similar cells at a later stage of development.

DESCRIPTION OF PLATES.

All the drawings were carefully traced with Abbé's drawing apparatus (Zeiss). The lenses used were the 3 mm. 1.40 N.A. apochromatic lens or the 4 mm. 0.95 N.A. apochromatic lens of Zeiss with compensating oculars 4, 6 or 8. The substage condenser was the achromatic combination 1.0 N.A. of Zeiss.

The following lettering in the figures used to indicate the different catagories of free cells :—

s.m., small mononuclear ; *l.m.*, large mononuclear ; *b.p.*, basophile polymorph ; *g.p.*, granular polymorph ; *f.g.p.*, finely granular polymorph ; *c.g.p.*, coarsely granular polymorph ; *ery.*, erythroblast.

PLATE I.

Fig. 1. Group of free cells in a space between the mass of yolk cells (*ly*) and splanchnic mesepithelium (*spm*). × 500. Stage 26. *Som.*, somatopleure.

Fig. 2. Primitive yoke-laden blood corpuscle from heart tube ; safranin. Stage 26. × 800.

- Fig. 3. Corpuscle from heart with fibrillar ring. Yolk stained black with iron hæmatoxylin. Stage 27, Series 84B. 2. $\times 800$.
- Fig. 4. Corpuscle from vessel on surface yolk in vertical section. Stage 27, Series 84B. 5. $\times 800$.
- Fig. 5. Corpuscle with centrosome and sphere from heart; fibrillæ of ring appear as equatorial groups of granules. Stage 27, Series 84B. 2. $\times 800$.
- Fig. 6. Large vacuolated ringed corpuscle from heart. Stage 30, Series 93C. 3. $\times 800$.
- Fig. 7. Giant corpuscle in vertical section from heart. Stage 30, Series 93C. 3. $\times 800$.
- Fig. 8. Small ringed yolk-free corpuscle with alveolar protoplasm and round nucleus. Stage 30, Series 93C. 3. $\times 800$.
- Fig. 9. Same in vertical section. Stage 30, Series 93C. 3. $\times 800$.
- Fig. 10. Corpuscle from heart, with centrosome sphere and lobed nucleus and without ring. Stage 30, Series 84B. 2. $\times 900$.
- Fig. 11. Giant apparently multinuclear cell from heart. Stage 30, Series 91C. 13. $\times 500$.
- Fig. 12. Small corpuscle with alveolar protoplasm faintly fibrillar. Stage 30, Series 93C. 9. $\times 800$.
- Figs. 13 and 14. Small corpuscles in vertical section, showing groups of granules at equatorial points. Stage 30, Series 93C. 5. $\times 800$.
- Fig. 15. Large cell with lobed nucleus. Stage 30, Series 93C. 5. $\times 800$.
- Fig. 16. Small cell apparently being budded off into vascular space (*v.*) on surface of yolk cells (*hy*). Stage 30, Series 93C. $\times 800$.
- Fig. 17. Cell with polymorphic nucleus centrosome and sphere in space on surface of yolk cells (*hy*). Stage 30, Series 93C. 4. $\times 500$.
- Fig. 18. Erythroblast with basophile protoplasm, and faint circumferential fibrillation. Nucleus with some closely-grouped, larger, and darker-staining karyosomes, and more distinct intervening reticulum. Stage 32+, Series 113C. 24. $\times 800$.
- Fig. 19. Same in vertical section. Stage 32, Series 113C. 35. $\times 800$.
- The character of the nucleus in both these cells is markedly different from that of the nucleus of the similar elements of earlier stages, figs. 13 and 14.

PLATE II.

- Fig. 20. Portion of splanchnic mesenchyme near its developing edge. Stage 31+, Series 106C. 11. $\times 400$. Below is mass of yolk cells (*hy*) with nuclei and yolk grains, and very indistinct cell outlines; above, the mesenchyme consisting of fixed and free cells, and showing spaces filled with blood corpuscles.
- Fig. 21. Section, through the mesonephros and adjoining parts. Stage 31+, Series 106C. 35. $\times 400$. To right, the notochord (N.), aorta (A.), and cardinal vein (C.V.); to left, the inner outline of the muscle plate (*m.p.*).
- Fig. 22. Section of the splanchnic mesenchyme at point M, text figure 2. Stage 32+, Series 113C. 22. $\times 500$. Below and to left is mass of yolk cells (*hy*), above is the line of the mesepithelium (*spm.*). The free cells of the mesenchyme have almost all become polymorphonuclear, and a number are coarsely granular.
- Fig. 23. Section through the mesenchyme between aorta and pronephric duct dorsal to cardinal vein (*cf.* text figure 4). Stage 32+, Series 113C. 34. $\times 700$. To left, outline of pronephric duct (P.D.); the section passes through an opening from the venous sinus round the duct into the cardinal vein (C.V.).

PLATE III.

- Fig. 24. Another portion of nephric tract of mesenchyme. Stage 32+, Series 113C. 34. $\times 600$. Below, a typical erythroblast (*ery.*); above and to the right, a large mononuclear (*lm.*), contrasting in size with the smaller polymorphonuclear cells.
- Fig. 25. Section through part of venous sinus round pronephric duct in region of pronephros. Stage 32+

Series 113C. 24. $\times 700$. Below and to right is pronephric duct (P.D.) The sinus is crossed by delicate protoplasmic strands with elongated nuclei. In the meshes of this reticulum, polymorphonuclear granular leucocytes (*gpc.*). In sinus space itself many large mononuclear cells, also erythrocytes drawn in outline, some of them in division.

Fig. 26. Section through mesonephros. Stage 32+, Series 113C. 40. $\times 500$. A, aorta; C.V., cardinal vein; P.D., pronephric duct. The nuclei of the tubules have not been filled in. The meshes of the reticular tissue are filled with leucocytes of all varieties.

Fig. 27. Section through splenic vein. Stage 36, Series 139. 19. $\times 300$. *a*, outline of stomach wall; *b*, spleen; *c*, liver. In vein, a polymorphonuclear leucocyte and four mononuclear cells.

PLATE IV.

Fig. 28. Section through spleen rudiment. Stage 32+, Series 113C. 22. $\times 400$. Below and to right, outline wall of stomach.

Fig. 29. Section through spleen rudiment. Stage 33, Series 108. 11. $\times 300$. Below and to right, wall of stomach, with its nuclei represented in outline. *a, a, a*, peripheral blood sinus.

Fig. 30. Section of spleen; Stage 37; Series 139. 20. $\times 200$. *s*, outline wall of stomach.

Fig. 31. High-power view of portion of same section, showing cellular trabeculae and spaces containing red blood corpuscles and white cells of different varieties. $\times 700$.

Fig. 32. Section of anterior end of spleen. Stage 39, Series 137B. 11. $\times 400$. Above and to left, vein (*spl.*); in centre of spleen, artery (*a*).

Fig. 33. Portion of same. $\times 850$. *a*, central artery; *b*, peripheral sinus. Four large cells in a row: to left, two large mononuclear spleen cells, *c*; to right, secondary erythroblast with hæmoglobin, *d*; in centre, primary erythroblast, *e*. Cells *f* and *g* were introduced from a neighbouring section; *f* has a nucleus like the spleen cells, *c*, but the basophile protoplasm shows delicate concentric fibrillation; *g* has a similar but deeply notched nucleus, and the protoplasm is very delicate and hyaline. It is probably a leucoblast.

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