Blood Cell Formation in Certain Teleost Fishes

By W. T. Catton, M.Sc.

The advances in our knowledge of blood-cell formation in the lower vertebrates have been due largely to Downey, and to Jordan and his associates. A comprehensive survey of the "lymphoid" tissue in the kidneys of many fishes was made by Drzewina, and a more detailed study was reported by Downey on the hematopoietic renal tissue of the spoonbill sturgeon, Polyodon spathula. On the basis of histologic examination of the kidneys of certain fishes, Jordan and Speidel presented a hypothesis as to the nature of the stem cells and the process of blood-cell development. They regarded the free stem cell in fishes as being similar to, and on morphologic grounds at least, as indistinguishable from a lymphocyte. This view seems in some degree to contradict the present working hypotheses of mammalian hematopoiesis. Of these, the most widely accepted are the monophyletic view, as propounded by Maximow and others, and the modified polyphyletic or dualist view based on the work of Doan, Cunningham and Sabin. In the monophyletic view there is a common stem cell, the "hemocytoblast," which differs in both size and structure from the lymphocyte. In the scheme of Doan, Cunningham and Sabin, there are two independent lines of development, the parent cell of the erythrocytes (termed by them the "megablast") arising as a daughter cell of the division of an endothelial cell of the wall of a marrow sinus, and the parent cell of the leukocyte series ("primitive white cell") arising extravascularly from a reticulum cell. It is evidently not possible to correlate either of these views with the accounts of Jordan and Speidel in reference to fishes.

Although there are no a priori grounds for denying that the blood stem cells may be of a different nature in fishes and mammals, representing extremes of the vertebrate scale, yet the commonly accepted view that the vertebrates form a compact phylogenetic group appears to render such a conception open to controversy. It was the aim of the present work to re-examine the question of the nature of the stem cells in teleost fishes in the light of the above considerations. Since no previous account of the cells of the circulating blood in the fishes studied (roach and trout) could be found in the literature, a preliminary survey of the blood of these fishes was made.

Materials and Methods

The material consisted of freshly caught specimens of English brown trout (Salmo trutta), common roach (Rutilus rutilus) and perch (Perca fluviatilis), taken from streams and reservoirs. Certain observations were also made on the marine teleosts, Ctenolabrus rupestris and Trigla cæcatus, during a visit to the Plymouth laboratory of the Marine Biological Association of the United Kingdom.

From the Department of Physiology, King's College, University of Durham, Newcastle-upon-Tyne, England.

A European member of the carp family.
The methods employed were mainly histologic and were generally similar to those described by Duthie in his work on marine teleost fishes. Fresh blood was obtained by stunning the fish by a sharp blow on the head and quickly amputating the tail. The blood was collected in waxed watch-glasses, with heparin or sodium citrate as anti-clotting agent. For microscopic examination of living cells, suitable dilutions were made in an isotonic saline described by Young. For supravital staining, mixtures of neutral red and janus green were prepared as follows. The stains were dissolved in absolute alcohol in each case to give a saturated solution; 20 to 30 drops of the stock neutral red solution were added to 10 ml. of absolute alcohol; to this were added 20 drops of janus green solution. The mixture was then flooded onto clean slides, which were stood on end to dry. In use, a drop of blood saline was placed on the slide and a coverslip added. Observations on surviving “kidney pulp” were made in the same way. For fixed stained films, the films were prepared in the usual way and allowed to dry in air for five minutes before fixation. This was found to give better results than fixation of “wet” films. Fixatives employed were methyl alcohol, the vapor of 40 per cent formalin and the vapor of 2 per cent osmium tetroxide. For the preservation of cytoplasmic inclusions, osmium tetroxide gave the best results; staining was not adversely affected, contrary to the usual view with regard to this fixative. Methyl alcohol and formalin vapor gave better nuclear fixation than osmium tetroxide, but were inferior as regards preservation of cytoplasmic structures. The fixed films were stained in a dilution of commercial Giemsa stain (Gurr’s R66), and were washed in water buffered to pH 7.2. For the peroxidase test the method of Sato and Sekiya was used. Material for sectioning was fixed in Zenker-formol as a routine. For some roach material, Regaud’s formol-bichromate was employed, as it was found to preserve the coarse granulocytes better than the routine fixative. After Regaud, sections were stained with Altmann’s acid fuchsin-methyl green method; after Zenker-formol the diluted Giemsa was used. In order to demonstrate the peritubular capillaries and structure of the renal intertubular tissue, injections of Indian ink in saline were made into the heart of narcotized fish. Specimens were killed at periods of up to twenty-four hours after injection and the kidneys then were removed for sectioning. Absolute red cell counts were made with the standard hemocytometer, using Young’s saline as diluting fluid. The pipets were previously treated with heparin to prevent clotting. Differential leukocyte counts were carried out in the usual way.

**Observations**

*The Cells of the Circulating Blood*

These consisted of nucleated erythrocytes, small and medium-sized lymphocytes, thrombocytes and coarse and fine granulocytes. Immature and also senile erythrocytes were commonly seen. The immature forms were distinguished by their circular outline and by the presence of a reticulum in their cytoplasm, which stained clearly with supravital cresyl blue, but could also be seen in the fixed stained films. The films were characterized by the presence of many more disintegrating cells and more cell debris than are usually seen in films of mammalian blood. Most of this fragmentary material appeared to be derived from the breaking down of erythrocytes and coarse granulocytes. Isolated disintegrating nuclei of erythrocytes, sometimes with part of the cytoplasm still attached, were scattered widely over the preparations. The appearance of these nuclei was quite different from that of the nuclei of normal cells, the chromatin being spread out in the form of an open network, whereas in the normal nucleus the chromatin material forms a compact mass. It was not possible to identify any cells resembling the mammalian monocyte, and it was decided to classify all medium sized non-granular leukocytes as medium lymphocytes. In fry and yearling trout, occasional
basophilic erythroblasts and early stem cells were found; in older fishes such precursor cells were not seen in the blood. It thus appears that in the adult fish the blood cells are released from the hematopoietic organs in a mature state, as is the case in mammals except for the reticulocyte stage of erythrocyte development.

**Erythrocytes** (fig. 1, a5). These number about 1,200,000 per cu. mm. in adult trout and about 1,500,000 per cu. mm. in adult roach. Considerable variation occurred in either species at the same season; counts in trout varied between 1,000,000 and 1,450,000, and in roach between 1,150,000 and 1,750,000. The average number in perch was about 1,750,000. The cells were oval in outline and biconvex in profile, the convexity being formed round the large central nucleus. When living, the cell showed a pale yellow color as viewed by transmitted light under the microscope. When suspended in isotonic saline, trout cells measured 14 /2 by 9 /2 and roach cells 16 µ by 10 µ. In fixed films the dimensions were reduced by shrinkage to about 11 µ by 8 µ in each species. With Giemsa stain the nuclei took on a deep mauve color and the cytoplasm a pale pink. Non-nucleated but otherwise normal cells were occasionally seen.

**Reticulocytes** (fig. 1, a4). These number about 1 to 2 per cent of the erythrocyte population. They were easily identified by cresyl blue stain supravitally, but could also be seen in the Giemsa-stained films and even in suspensions of blood in saline, being then distinguished by their circular outline. The reticulum could be recognized in the fixed stained films, but presented a different appearance from that seen in supravitally stained blood. Reticulocytes measured about 11 µ in diameter in the living state.

**Thrombocytes.** (fig. 1, a7). These numbered about 2,000 per cu. mm. in roach and trout, and they varied in size from 7 µ by 4 µ to 10 µ by 4 µ. The cells were flask-shaped, with a pointed projection at one or both ends, as seen both in fixed films and in living blood. The nucleus resembled the erythrocyte nucleus, and the cytoplasm took on the same shade of pink as that of the erythrocyte, suggesting that the thrombocyte may also contain hemoglobin. No such cells could be found in the blood of perch.

**Lymphocytes** (fig. 4, 3). These numbered from 20,000 to 40,000 per cu. mm. and varied in size from 5 µ to 10 µ in diameter in both roach and trout. They resembled the mammalian lymphocyte in appearance; occasional cells were seen to contain large discrete eosinophilic granules in the cytoplasm, (fig. 1, m), an anomaly already described in mammalian lymphocytes. These are the cells which are drawn and described by Lanine,14 and which he considered to be the "eosinophil" cells of fishes.

**Fine granulocytes** (fig. 1, b2-f2, g). These numbered from 3,000 to 6,000 per cu. mm. In living preparations of trout blood they measured 8 µ to 10 µ in the inactive state, and reached 20 µ in length when extended in ameboid motion. In fixed films the size ranged from 9 µ to 12 µ. In the roach the corresponding cells measured from 10 µ when inactive to 24 µ when extended. The cells were usually to be found adhering to the undersurface of the coverslip, where their movements could most readily be observed. Single blunt pseudopodia were produced, and the inner
FIG. 1.—See legend, opposite page
cytoplasm flowed freely, as in Amoeba, the fine protoplasmic granules showing Brownian movements. With neutral red-Janus green supravital staining, the characteristic granules appeared pale brown, and interspersed between them were numerous finer particles, stained pale green; these may have been mitochondria. The nucleus was undivided or only slightly constricted into two lobes, in the roach cells; in trout cells it was dissected into 3 or 4 lobes, connected by fine bridges as in the mammalian polymorph neutrophil cell. The fine cytoplasmic granules were seen most clearly in sectioned material of kidney, gill, and intestine, where they were stained with eosin. In fixed stained films the granules were unstained with normal staining times, but took up eosin after prolonged staining. In roach
and trout the granules gave a positive peroxidase reaction. In the fishes examined, fine granulocytes were found only in the circulating blood and hematopoietic centers, and not in the tissues generally. It is concluded that they serve the same role, as scavenging phagocytes, as the mammalian neutrophil cells, which they so closely resemble in structure.

**Coarse granulocytes** (fig. 1, b-f3, b, i; fig. 4, 3). These cells were rarely seen in films of circulating blood of the freshwater species examined; they occurred fairly frequently in *Ctenolabrus* blood films, but rarely in films of *Trigla* blood. The scarcity of this type of cell in the blood films of fishes was observed by many previous workers, and some were led to assume that coarse granulocytes were entirely absent from the blood. If living blood is examined however, these cells can readily be seen, and when stained with neutral red supravitaly the granules take on a yellowish-brown color. Differential counting of the cells in fresh blood is rendered difficult by the active movements of the cells, but by comparison with the numbers of erythrocytes in the same fields an average number of 100 to 500 per cu. mm. was estimated. In contrast with the scarcity of the cells in fixed blood films, they were found in large numbers in the hematopoietic centers (kidney and spleen), in the intestinal submucosa, (fig. 4, 4), and in the gill lamellae, (fig. 2, 2). They were also found in the mesenteries and peritoneal fluid. The scarcity of coarse granulocytes in the blood films is probably to be explained by their fragility; coarse granules were to be seen scattered throughout the films, as also were fragments of the cells themselves. In living blood of trout the cells measured from 9 to 11 μ when subspherical in shape (inactive), and reached 18 μ in length when extended in ameboid motion. They adhered to the underside of the coverslip in the same way as the fine granulocytes. The granules measure about 1 μ in diameter and float freely in the fluid "endoplasm" of the cell. In fixed stained films of trout blood the coarse granulocytes presented an appearance difficult to interpret. In the cytoplasm was a mixture of granules of varying size, and also showing different staining characteristics. Most of the granules were of the larger sizes, and these were stained blue. The finer granules, interspersed among them, appeared mauve in color, and the smallest of them appeared crimson. The apparent color differences may be open to a purely physical interpretation, as being due to the diffraction of the light passing round the small particles. The smallest particles are of the order of size of mitochondria, which are not, however, commonly reported as staining with any component of the Romanowsky stain employed. In roach blood, the coarse granular cells were rarely seen in fixed films; when so preserved, their granules were stained mauve in color. The staining reaction of the granules is thus basophilic in trout, and metachromatic in roach. The coarse granular cells of *Trigla* were found to be basophil and those of *Ctenolabrus* acidophil, in agreement with Duthie. In the perch, the coarse granulocytes, as observed in the spleen, intestine and gill were acidophil.

**Granular anucleate bodies.** (fig. 1, n). Small rounded masses, 5 μ in diameter, containing a mixture of basophil and acidophil granules in a pale blue "cytoplasm," were seen frequently in blood films of roach and trout. They were at first considered to be fragments of broken down coarse granulocytes, but they regularly showed
such smooth unbroken outlines and relative uniformity of size as to suggest that they were morphologic entities. In no case was a nucleus seen in any of these bodies. No previous report on these structures has been found in the literature.
Discharge Pattern of the Coarse Granulocytes

At various limiting membranes, such as the intestinal mucosa, peritoneal epithelium and gill epithelium, the coarse granulocytes of roach, trout and perch were observed in a condition similar to that described by Duthie in marine fishes. The cell is seen with its long axis perpendicular to the epithelial surface, with the nucleus lying at the pole distant from the surface (fig. 2, 7 and 3). The granules, still retaining their characteristic staining, are seen to have become elongated and club shaped, and to be arranged so that they appear to converge at the apex of the cell, on a level with the surface of the epithelium. This appearance suggests that the granules were in the process of discharging a fluid at the surface. In every case observed the granules presented the same appearance and were all of equal size; there was no suggestion that each granule was dissolving as a whole, but rather that a fluid was being discharged from it, leaving a permanent envelope within the cell. It is proposed that the granules of these cells are of the nature of small vesicles, consisting of an envelope with a content of fluid. Some slight evidence in favor of this view may be quoted. First, that the granules give a positive result with the Smith-Dietrich test for lipoid material (Al-Hussaini, private communication), as has been shown for the envelopes of erythrocytes. Second, that in certain mammals in which the eosinophil cells have been observed in the intestinal mucosa, the ‘granules’ appear to have swollen to two to three times the size in which they appear in the blood stream (Catton, unpublished data).

The coarse granulocyte of these fishes, in its structure and habit of migrating from the capillaries into surrounding tissue, bears evident resemblance to the
mammalian eosinophil cell. I have been unable, however, to find examples of the discharge pattern phenomenon described above in any mammalian tissue examined. Downey\(^6\) ascribed secretory activity to all the granular leukocytes in the ganoid, *Polyodon spathula*, and was led to assume that the granules were a specific secretion of the cells, which was liberated in their passage through the circulation. He classified these cells into three main 'series,' based on the morphology as revealed by staining with safranin and toluidin blue. It is difficult to correlate his classification with that of more recent reports. He refers to Stephan\(^9\) who in 1906 described cyclic changes in the size of the granules in the granular leukocytes of *protoperus* (Dipnoi). In hibernation, the granules gradually shrank. In the active season, on recommencing feeding, the granules became enlarged.

### The Sites of Hematopoiesis

The chief sites of hematopoiesis in these fishes were in the kidney and spleen. In the roach, as in *Ctenolabrus* and *Trigla*, only the kidney shows activity. In the perch, activity is limited to the spleen. In the trout, spleen and kidney are both active. The presence of hematopoietic tissue in the kidney may be detected macroscopically by the intense red color of the organ, or of such parts of it which are hematopoietically active. The distribution of active zones in the kidneys of some teleost fishes studied is shown in figure 3. The distribution was found to be similar in members of the same species.

### The Renal Intertubular Hematopoietic Tissue

This is the tissue referred to by Drzewina\(^7\) as 'lymphoid', in a general survey of the 'lymphoid' tissue of fishes. It was studied in more detail by Downey\(^6\) in the kidney of a ganoid, *Polyodon spathula*. When examined microscopically, the hematopoietic activity is seen to be taking place in this tissue, which presents a similar degree of complexity to that of red bone marrow. It is first clearly seen that a capillary network surrounds each kidney tubule; in noninjected specimens, these capillaries were recognized by the epithelial lining cells and the presence of blood cells within them. After the injection of india ink into the circulation, the capillaries were very clearly demonstrated, the ink particles adhering to the inner surfaces of the lining cells and also possibly being ingested by them (fig. 4, i). At certain points along the capillaries the ink was seen to follow narrow channels of escape, which communicated with general spaces within the intertubular tissue, and many particles were taken up by macrophages scattered throughout this tissue (fig. 1, k). In specimens in which the kidney was removed for fixation within fifteen minutes of injecting of ink into the heart, the macrophages had already taken up the ink particles, indicating a free circulation of blood within the intertubular tissue. The nature of this circulation was very difficult to follow, owing to the dense crowding of the blood cells and their precursor elements. Strands of reticular cells were observed, and these in places formed close meshes round the developing blood cells, so that a general picture was deduced of a 'spongework' of reticular cells extending between the peritubular capillary nets, and forming the supporting frame of the hematopoietic
FIG. 4.—See legend, opposite page

48
tissue. In a few parts of each section, however, parallel chains of endothelial cells could be seen to enclose channels which contained mainly normal blood cells. The endothelial walls of these spaces were always incomplete.

The spaces so defined might be regarded as primitive representatives of the "open" sinuses in the bone marrow, described by Doan; it was not possible to identify any structures corresponding to the "closed" or "formative" sinuses of Doan, and as far as could be seen the blood-forming cells lie in masses retained in the reticular spongework of the intertubular tissue. There was no evidence of segregation of the precursor cells of erythrocytes and granulocytes, these immature cells being observed closely intermingled with each other and bearing no well defined relation to the incomplete sinuses described above. The appearance of the intertubular tissue is represented in the microphotograph (fig. 4, 2), and by the simplified diagram, (fig. 2, i).

In fishes in which the kidney was not active in blood-cell formation (as in perch), the reticular stroma was found to be absent also, the tubules being then more closely approximated, and separated only by the capillary networks surrounding each one of them. This suggests that the reticular stroma is not necessarily present only as a supporting tissue, but may serve the additional function, in the hematopoietically active kidney, of providing the blood stem cells.

Regarding the circulation of the intertubular tissue, Downey showed by injecting a gelatin mass into the caudal vein, that renal portal blood entered into the channels and spaces in the tissue. The gelatin did not pass any further into the vascular system. My india ink injections into living fish by an arterial route have shown channels of communication between the peritubular capillaries and these spaces. It is to be assumed, then, that the blood flows normally from the renal portal veins, through the intertubular tissue, and thence into the peritubular capillaries, finally emerging in the renal veins.

Groups of cells, having the character of chromaffine cells, were observed to be scattered throughout the intertubular tissue. In unstained sections, these cells were seen to have retained a strong brown color, suggesting an affinity for the bichromate of the fixative (Zenker-formol). Each of these cells contained three to six large clumps of a substance which took a green color after staining with Giemsa, (fig. 1, j). The green color could be ascribed to a superposition of the blue component of the stain on the brown color due to the bichromate fixation. These

---

Fig. 4.—1. Roach kidney, sectioned after injection of india ink. Fixed Helly, stained Giemsa. × 345. Ink particles in peritubular capillaries.
2. Ctenolabrus kidney. Section 5a, fixed Helly, stained Giemsa. Intertubular tissue. × 345.
cells were identified in films and sections of kidneys of trout, roach, *Trigla* and *Ctenolabrus* (fig. 4, 5).

**The Phylogeny of the Blood Cells**

The view is now widely accepted that the blood cells take their origin from reticulo-endothelial tissue as found in the hematopoietic organs, this tissue being a remnant of the undifferentiated mesenchyme surviving from embryonic life. These cells retain the potency for further differentiation, which is lost in the specialized tissues of the body. The first step in blood-cell formation is the renewal of activity in some of these cells, which undergo certain morphologic changes. These cells may then either become detached from the parent tissue and form "free" stem cells, or may remain attached, as "fixed" stem cells, and give rise to "free" stem cells by mitotic divisions. This process is notably difficult to follow in the study of mammalian bone marrow, and has proved no less so in the study of hematopoiesis in fishes. Evidence of a transformation of a "fixed" reticulum cell into a "free" stem cell was found on two occasions only, in the study of many sections of the kidneys of different fishes. In these two cases, reticulum cells were observed, still forming part of the reticular stroma, which had undergone morphologic changes approaching the structure of a free stem cell. These changes were as follows: a considerable increase in size of both the nucleus and the cell as a whole; a markedly increased basophilia of the cytoplasm; and the development of the prominent chromatin "knots" in the nucleus, typical of the free stem cell to be described, when seen in sectioned material (fig. 5).

The rarity with which such transformations were observed might imply (a) that the phenomenon is difficult to observe in the cell-crowded intertubular tissue, or (b) that it is a rare occurrence in adult fishes; evidence in favor of the latter view will be given later.

The free stem cell so derived is a large cell of lymphoid character, reaching 15 μ in diameter, subspherical in shape and possessing a large nucleus and a narrow peripheral zone of cytoplasm, which is intensely basophilic, (fig. 1, d, e). In fixed films, many cells of the same general appearance were seen, ranging in size from 7 μ to 15 μ. There was a marked difference in the appearance of these cells as between those seen in films and in sections. In the films, the nucleus showed a uniform network of fine chromatin strands, with a thin nuclear membrane and no evident nucleoli; fine basophil granules were seen in the cytoplasm. In sections, the nuclear chromatin was seen to be condensed into one or two "knots," possibly around nucleoli not otherwise visible, and the rest of the nucleus appeared to be devoid of solid structure, (fig. 1, e). It is reasonable to suppose that this knotting of the nuclear chromatin is a fixation artefact, and that we see a truer picture of the cells in film preparations. Stages of mitosis were seen in cells of this type, both in films and sections, but far more clearly in the former. Mitoses were more frequently observed in the larger cells than in the smaller ones. The most common stages seen were metaphases, (fig. 1, i), and telophases.

Regarding the part played by these lymphoid cells in the process of hematopoiesis, from a histologic study alone it is only possible to postulate hypothetical
Fig. 5.—Scheme of development of blood cells in Teleost fishes (diagrammatic). (B indicates basophilic, the plus signs indicating increased basophilia. A indicates faintly acidophilic. P+ indicates peroxidase positive. P− indicates peroxidase negative. P (Gran.) indicates peroxidase positive, granules only. (a) Reticulo-endothelial cell. (b) Blood stem cell developed from R-E cell. (c) Detached blood stem cell (large lymphoid hemoblast). (d) Division of large lymphoid hemoblast. (e) Small lymphoid hemoblasts. (f) Mitosis of early erythroblast. (g) Erythroblast. (h) Reticuloeyte. (i) Erythrocyte. (j) Coarse progranuloeyte. (k) Fine progranuloeyte. (l and m) Progranuloeyte mitoses. (n and o) Mature coarse and fine granuloeytes. (p) Lymphoeytes. (q) Thrombocyte.

activity, and to follow the classic method of arranging the cells observed into series representing the most probable lines of development. I present my observations and deductions as follows.

1. Cells of a lymphoid character are seen in a range of sizes from 7 μ to 15 μ diameter.

2. The reticular stroma may give rise to the larger cells (two cases observed to
show transitional forms); but the possibility of cells of any size being derived in this way cannot be excluded.

3. It is reasonable to suppose that the smaller type of cell may grow into a larger one, but that the larger type will not develop into a smaller one by decrease in size.

4. Mitoses are more commonly seen in the larger cells, by casual observation; this does not necessarily imply a higher rate of mitosis.

5. In subsequent development, as described below, the large cells give rise to granulocytes, the small ones to erythrocytes, thrombocytes, and lymphocytes. At the extremes of the size range then, we have cell populations which differ not only in size, but also in developmental potentialities.

6. It is convenient to designate cells of the largest size as large lymphoid hemoblasts; those of the smallest size as small lymphoid hemoblasts.

7. It may be deduced that the population of each type of hemoblast is maintained by continuous mitotic divisions, and that derivation of free stem cells by 'recruitment' from the reticular stroma is not a normal process in the adult fish. This would explain the rarity with which this process could be observed in the sections.

The Development of the Erythrocytes (fig. 1, a1-a7)

The erythrocyte arises from a small lymphoid hemoblast, in which marked changes occur in both nucleus and cytoplasm. The nuclear chromatin becomes gradually more condensed as development proceeds, and at one stage there is a 'cartwheel' appearance of the peripheral zone of the nucleus (fig. 1, a3), such as has been described in late erythroblasts in mammals. The ratio of cytoplasm to nucleus increases steadily, with increase in size of the cell as a whole, which retains a circular outline up to and including the reticulocyte stage. The intense basophilia of the stem cell cytoplasm gives way to polychromatophilia in the erythroblast (a3), and this to acidophilia in the mature erythrocyte. The stages of development may provisionally be designated as small lymphoid hemoblast; proerythroblast (faintly basophil); erythroblast (polychromatophil) with cartwheel chromatin strands; reticulocyte; erythrocyte. Release into the circulation occurs at the reticulocyte stage. The development of the erythrocyte was most clearly followed in films of trout and roach kidney, but similar stages were made out in Ctenolabrus and Trigla preparations. Mitoses were frequently seen in proerythroblasts and erythroblasts, suggesting that the population of these cells was maintained to some extent by a process of homoplastic maintenance, in addition to heteroplastic derivation from the stem cells.

The Development of the Leukocytes

1. Granulocytes. Complete series of transitional stages linking fine and coarse granulocytes with large lymphoid hemoblasts were made out in all the species studied. The earliest stages showed the presence of a relatively few granules, situated in the cytoplasm in the region of a slight indentation of the nucleus. In later stages the granules had increased in number, the nucleus was smaller and
displaced to the periphery of the cell, and the cytoplasm was less strongly basophilic than that of the parent hemoblast. Mature granulocytes, as seen in peripheral blood, showed complete absence of basophilia. The maturing forms were termed 'progranulocytes' by Duthie,9 who described the maturation of these cells in some marine teleost fishes.

2. Lymphocytes. It was usually difficult to distinguish between blood lymphocytes and small lymphoid hemoblasts in films of kidney and spleen. Lymphocytes in the circulating blood showed a less basophilic and more translucent cytoplasm than that of small hemoblasts from the kidney; the nucleus also showed a denser appearance, with more compacted chromatin. In differential counting, no attempt was made to distinguish the two cells. It seems reasonable to assume that lymphocytes are derived from the small hemoblast population, with but slight change in morphologic structure.

Differential counts of kidney and spleen films of trout were carried out, and the results appear in table 1. It will be seen that in the kidneys of 3 year old fishes there is a pronounced reduction in the proportion of large lymphoid hemoblasts as compared to the 1 year old fishes, while the erythroblasts and small lymphoid cells remain at a fairly constant level. This seems to indicate a decrease in heteroplastic derivation of maturing cells from stem cells, with a relative increase of homoplastic maintenance among erythroblasts and progranulocytes in these older fishes. In the spleen, small lymphoid cells are seen to occur in greater proportion, and large lymphoid cells and erythroblasts in lesser proportion than in the kidney of the 1 year old fish.

**Discussion**

The following discussion does not attempt to be exhaustive, but to present such views extracted from the wide field of hematologic literature which seem closely relevant to the argument.

The earlier workers on the blood and blood-forming process in fishes (including Rawitz,16 Drzewina,7,8 Laninetm4) limited their descriptions mainly to observations on the cells of the circulating blood, and in particular to the coarse granulocytes.14

---

**Table 1.—Differential Counts of Kidney and Spleen Films of Trout**

<table>
<thead>
<tr>
<th>Cell-Type</th>
<th>1 year Trout kidney</th>
<th>3 year Trout kidney</th>
<th>1 year Trout spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large lymphoid hemoblast</td>
<td>33</td>
<td>12.5</td>
<td>20</td>
</tr>
<tr>
<td>Fine progranulocyte</td>
<td>15</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>Coarse progranulocyte</td>
<td>2</td>
<td>0.5</td>
<td>1.6</td>
</tr>
<tr>
<td>Fine granulocyte</td>
<td>10</td>
<td>17.5</td>
<td>6</td>
</tr>
<tr>
<td>Coarse granulocyte</td>
<td>0.4</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Erythroblast</td>
<td>10</td>
<td>12.5</td>
<td>5</td>
</tr>
<tr>
<td>Small lymphoid cells (including lymphocytes)</td>
<td>19.6</td>
<td>36</td>
<td>51.2</td>
</tr>
</tbody>
</table>

Figures are percentages of cell-populations of fixed films, excluding erythrocytes, reticulocytes and thrombocytes, and are averages of six counts on three slides from each specimen.
Drzewina described so-called lymphoid tissue in the kidney, spleen and other organs of certain teleost fishes, to which she ascribed the functions of lymphocyte and granulocyte formation. A later paper by the same author described the circulating blood cells of 68 species of fishes, and in particular drew attention to the variability in staining character of the coarse granulocytes.

Downey made a careful study of the intertubular hematopoietic tissue in the kidney of the spoonbill sturgeon, Polyodon spathula. His paper dealt mainly with the nature of the circulation through the tissue, the structure of the supporting reticulum, and a detailed analysis of the granulocyte population. He concluded that blood from the portal veins passed directly into 'lymphoid' tissue which surrounded the branches of these veins. Blood might also pass directly into a system of blood spaces lying in the meshes of the general reticular framework, and thence into the 'efferent vein.' These spaces he did not observe to be lined with endothelium. Blood which had passed through the lymphoid tissue flowed into these same spaces on its way to the efferent veins. I have not been able to trace an arrangement of this kind in the kidneys of the teleosts I have studied, but have found evidence of channels which are partly lined with endothelium, and of direct communications from the peritubular capillaries to the general spaces in the reticular framework. Downey adopted a monophyletic view of the origin of the blood cells, and described the original parent cell as a 'large basophilic mononuclear cell.' This cell appears to correspond to my large lymphoid hemoblast. Jordan and Speidel described in detail the varieties of cells found in the hematopoietic centers of the kidney and spleen in a number of elasmobranchs and teleosts. They described large and small 'lymphoid cells,' which they referred to in earlier works as 'lymphocytes,' in later works as 'lymphoid hemoblasts'; they regarded these cells as being functionally and morphologically identical with the blood lymphocyte. The small lymphocytes were said to develop into thrombocytes, the larger ones into erythrocytes, special leukocytes (fine granulocytes) and eosinophils (coarse granulocytes). The small lymphocytes were claimed to be derived from reticulum cells and to grow into large lymphocytes, which subsequently underwent amitotic division, the daughter cells then reinforcing the small lymphocyte population. The amitotic divisions were observed in fixed sectioned material, and their figures resemble the appearance of similar sections prepared by myself (fig. 2, i). It is doubtful whether they represent true amitotic divisions, since in fixed smears, evident true mitoses are seen to occur in these cells.

In a paper dealing with the morphology of the spleen in fishes, Yoffey gave an account of blood cell formation in this organ, with particular reference to the dogfish (Scyliorhinus canicula). He referred to observations on the spleens of some marine teleosts, but did not study any freshwater fishes. In a description of the cells observed in spleen smears of the dogfish he mentioned two types of cell which are of special interest. These cells he termed 'small round cells' and 'large round cells,' and from his detailed descriptions they appear to correspond with the small and large lymphoid hemoblasts described in this paper. He also noted a complete series of transitional forms leading from one type to the other.
His observations led him to adopt a monophyletic view, and he put forward a scheme of blood cell development as occurring in both elasmobranch and teleost spleens, which is the same as that of Bryce for postlarval forms of *Lepidosiren paradoxa* (Dipnoi). In this scheme the common ancestor of the blood cells is the "small round cell"; it undergoes transformation first into an erythroblast, and this in turn into an erythrocyte; this small cell may also grow into a large round cell, and from this type the granulocytes develop. Yoffey is thus in general agreement with Jordan and Speidel, except that he does not make the assumption that the hemoblasts (small round cells) are necessarily identical with blood lymphocytes.

Duthie9 described blood cell formation in certain marine teleost fishes, mainly of the families *Triglidae* and *Labridae*. He found the same varieties of large and small lymphoid cells as those described by Jordan and Speidel, but he termed the large cells 'Granuloblasts', since he deduced that they developed into granulocytes; the small cells he termed lymphoid hemoblasts, and he regarded them as the common stem cells. He concluded that the small lymphoid cells grew into the larger forms, and believed but was not fully convinced that they also developed into erythrocytes. He described karyokinetic figures in the lymphoid cells, more particularly in the smaller ones, and did not comment on the claim of Jordan and Speidel that the divisions of these cells were mainly amitotic. Drzewina had earlier claimed that both mitotic and amitotic divisions were to be found in the lymphoid cells of the renal hematopoietic tissue of fishes.

The view that the large lymphoid hemoblast is the original stem cell in the blood-cell-forming process of these fishes, as expressed in this account, is based upon the following considerations. First, that its nuclear structure bears a closer resemblance to that of the parent mesenchymal cells (in particular the reticulum cells) than that of any other possible formative cells found in the blood-forming tissues. Second, that stages have been observed which show a transition between apparent reticulum cells and this type of cell. Third, that these large lymphoid cells appear to undergo frequent mitotic divisions, by which the small lymphoid hemoblasts may be derived. Fourth, that the large lymphoid hemoblast bears a close resemblance to the "hemocytoblast" described by Maximow, Ferrata, and the monophyletic school as the single stem cell in mammals. It may be regarded as significant also that in the formation of granulocytes the first appearance of granules is seen in these large cells and not in the small ones. Small cells possessing granules occur commonly, but they are of the same general appearance as the mature granulocytes, in that the cytoplasm is almost filled with characteristically-staining granules; they could best be regarded, then, as division products of late stages in granulocyte maturation. If, according to previous authors, the small lymphoid cell were to develop into granulocytes, it might do so in one of two ways. First, by an increase in size with simultaneous formation of granules, up to the size of the large hemoblast; this is denied by the absence of intermediate stages. Second, it might first increase in size, delaying the formation of granules until it reached the size of a large hemoblast. It would be difficult then to explain this delay in granule formation, since the small lymphoid cell is sufficiently mature.
to be capable of direct morphologic change into an erythroblast, i.e., it is able to 
organize a profound change in cytoplasmic activity in this respect. Again, as-
suming the small lymphoid cell to act as the original stem cell, it would be re-
quired first to grow to the size of a large lymphoid hemoblast and subsequently 
for a decrease in size to occur to that of the mature granulocyte.

An alternative method of derivation of the small lymphoid hemoblast might 
be assumed to be as one product of the division of an endothelial-type cell, in a 
manner comparable with the derivation of the "megaloblasts" (proerythroblasts) 
from endothelial cells of the marrow sinuses in mammals and birds (Doan, Cun-
ningham and Sabin'). On this basis, the small lymphoid hemoblast would be 
homologous with the "megaloblast," and the large lymphoid hemoblast, derived 
from a reticular cell, would be homologous with the "primitive white cell" of 
the same authors. This would establish two independent lines of cells, culminat-
ing in the production of erythrocytes on the one hand, and of granular leukocytes on 
the other in both teleosts and higher vertebrates. There is, however, no evidence 
that small lymphoid hemoblasts are derived from endothelial cells in teleost fishes.

Jordan and Speidel' described the origin of "small lymphocytes" from reticulum 
cells; in a later work, they specifically stated that they did not observe the der-
ivation of "hemoblasts" from endothelial cells in the horned toad, phrynosoma 
(a reptile), but only from reticular cells. Duthie9 does not raise the question of the 
derivation of the stem cells and describes only their subsequent development.

The proposed schemes of blood cell formation discussed above are summarized 
as in figure 6, and the first scheme is shown diagrammatically in figure 5.

Attempts to elucidate the pattern of blood cell formation in mammalian and 
avian bone marrow have given rise to a number of different hypotheses, of which 
two of the most widely accepted are those proposed by Maximow15 and by Doan, 
Cunningham and Sabin.4 The latter workers, employing technics of artificially 
depleting and thereby simplifying the appearance of the bone marrow of pigeons 
by starvation, and of supravital staining of the marrow cells, elaborated a theory 
according to which two chief types of stem cell occur. One of these types ("megal-
blast") is said to arise from endothelial elements, and to develop inside "forma-
tive" sinuses in the marrow, giving rise to erythrocytes; the other type of stem 
cell is said to arise outside the sinuses, is referred to as the "primitive white 
cell," and gives rise to the granular leukocytes. On this theory, the erythrocytes 
have an "intravascular" origin and the leukocytes an "extravascular" origin, and 
further they develop from distinct types of stem cell, whose morphologic 
differences are best made out by supravital staining. Such a theory is included in 
the general term "polyphyletic."

The older ("monophyletic") school, whose views are summarized by Maxi-
mow,16 maintained that all blood cells arose from a population of morphologically 
identical stem cells ("hemocytoblasts"), but that the subsequent lines of de-
velopment were decided by the nature of the environment in which the stem cell 
first arose. Thus stem cells (medium lymphocytes) arising in the lymph glands 
developed into lymphocytes, while morphologically similar cells arising in the 
bone marrow sinuses gave rise to erythrocytes, and those outside the sinuses to
granular leukocytes. Maximow also believed that the lymphocyte was capable of developing into all types of blood cells; his "hemocytoblast" appears to correspond to Jordan's large lymphocyte and in a more limited way with the large lymphoid hemoblast described in this work. Jordan produced evidence in support of the view that the environment of the developing stem cell (lymphocyte).

**Jordan and Speidel** (1924)

- Reticulum cell of reticulo-endothelium
- Lymphoid hemoblast (small lymphocyte)
  - Small lymphoid hemoblast
  - Medium lymphoid hemoblast
  - Large lymphoid hemoblast
    - Blood lymphocytes
    - Thrombocytes
    - Erythrocytes
    - Granulocytes

**Dutrie (1935)**

- Lymphoid hemoblasts
  - Erythroblasts
  - Granuloblasts
  - Reticulocytes
  - Fine progranulocytes
  - Coarse granulocytes
  - Blood lymphocytes
  - Thrombocytes
  - Erythrocytes

**Present Author**

- Reticulo-endothelium
  - Endothelial cells
  - Small lymphoid hemoblasts
  - Large lymphoid hemoblasts
    - Blood lymphocytes
    - Thrombocytes
    - Erythroblasts
    - Reticulocytes
    - Erythrocytes

Fig. 6.—Proposed schemes of blood cell formation.

exerts a decisive influence on its subsequent development. In *Diemyctylus* he found that cells of identical appearance in spleen and liver capsule gave rise to erythrocytes and granulocytes respectively.

Those workers who were not convinced of the possibility of the environment having such a decisive effect on the developing stem cells proposed a modification of the original monophyletic (unitarian) theory. The divergence of opinion arose
mainly over the question of the possible identity of the marrow lymphoid cells (myeloblasts) with the large lymphocytes of the lymph nodes. Certain authors, commencing with Naegeli, have maintained that these cells were distinct, the myeloblast giving rise to all the blood cells excepting lymphocytes, the large lymphoid cells of the lymph nodes being responsible for lymphocyte production only, under normal conditions. Maximow repeatedly failed to find consistent differences between the lymphoid cells of the marrow and lymph nodes; such differences that he could detect he ascribed to the effects of the different environments. The neo-unitarian theory (Naegeli, Pappenheim, Ferrata, Downey) seeks to maintain the distinction between the marrow lymphoid cells (myeloblasts or lymphoidocytes) and the lymphoid stem cells of the lymph nodes, although under normal conditions few stem cells are believed to be active, the lymphocyte population being maintained homoplastically. This functional dualism breaks down under pathologic conditions, and all blood cells, including lymphocytes, are derived from the myeloblast.

The monophyletic school, then, assumes that there is only one type of stem cell, which is inherently pluripotential, and that the subsequent course of its development is dependent on the environment in which it arises (marrow or lymph node). The neo-unitarian school distinguishes two types of stem cell, the myeloblast and the precursor cell of the lymphocyte, each under normal conditions having its own limited potentiality of development, the environment having no particular significance.

The work of Doan et al. presents a morphologic picture of the marrow in which reasonably adequate segregation of distinction "physiologic environments" may be considered as a possibility, these being the environments afforded by the interior and exterior of the sinuses respectively. But in teleost fishes it has been shown in this investigation that the renal intertubular tissue offers no evidence of discrete sinuses or of formative pockets limited to one type of cell; on the other hand the developing cells appear to lie closely intermingled, precursor cells of both the erythrocytic and granular leukocytic series lying alongside each other. It is difficult then to conceive any differential effects on the developing cells.

The scheme of blood-cell development in fishes proposed in this work does not fit closely with any of the accepted views on mammalian hematopoiesis, but approaches most closely to the neo-unitarian hypothesis, in the light of the following considerations. First, that two types of stem cell are recognized, the large and small lymphoid hemoblasts, which differ from each other, however, only in size. Second, owing to the difficulty of prescribing segregated environments for the two types of cell, it must be assumed that each has an inherently limited potentiality of further development, in that distinct lines of development are pursued by the cells lying within the same environment. The large lymphoid hemoblast resembles the mammalian myeloblast in size and appearance, but in the fishes examined gives rise only to granulocytes directly, all other cells (including lymphocytes) arising from the small lymphoid hemoblast. Nor can this cell be closely homologized with the hemocytoblast of the monophyletic school, since only indirectly is it the stem cell for erythrocytes, lymphocytes and thrombocytes.
If evidence can be found of the derivation of the small lymphoid hemoblasts described, from endothelial cells, we should have justification for a polyphyletic theory, and be able to correlate the blood-cell forming process in teleosts with that in birds and mammals, according to the theory of Doan, Cunningham and Sabin.

**Summary**

1. The blood cells of trout and roach consist of nucleated erythrocytes and reticulocytes, nucleated thrombocytes, coarse and fine granulocytes, and lymphocytes of varying sizes. It is difficult to distinguish any cells showing characteristics similar to those of the monocytes of mammals. Immature cells occur more frequently in the blood than is the case in mammals.

2. The coarse granulocytes very commonly escape from the blood vessels, and have been observed in large numbers in the intestinal mucosa and submucosa, in the gill epithelia, and in the peritoneum. These cells migrate to the epithelial surface, where they undergo changes in structure leading to the formation of a characteristic discharge pattern of their granules. It is proposed that the “granules” are in reality vesicles with fluid contents, which are ultimately discharged at epithelial surfaces.

3. The hematopoietic organs of these fishes are chiefly the intertubular tissues of the kidneys; in the trout the spleen is also active; in the roach, only the kidney is active; in the perch, only the spleen is active.

4. Two alternative hypotheses of blood cell formation are proposed. On the first hypothesis, the common stem cell is described as a “large lymphoid hemoblast,” which gives rise to granulocytes by direct transformation and undergoes mitotic division to give rise to “small lymphoid hemoblasts.” From the latter develop the erythrocytes, thrombocytes and blood lymphocytes.

On the second hypothesis, the large lymphoid hemoblast, derived by transformation of reticular cells is the precursor solely of the granulocytes, and the small lymphoid hemoblast is to be derived from endothelial cells and is the precursor of erythrocytes and thrombocytes. In this case the large cell is to be compared with the “primitive white cell” of Doan, Cunningham and Sabin, and the small cell with the “megaloblast” of the same authors. No evidence however is available of the derivation of small hemoblasts from endothelial cell components of the reticulo-endothelium.

5. In the maturation of the erythrocyte in teleost fishes, there is a progressive increase in the size of the cell; in mammals and birds there is a decrease in size. In both cases there is a decrease in size in granulocyte maturation.

6. There are no essential differences between the blood cells and hematopoietic processes of the freshwater and marine teleost fishes examined.

**Acknowledgments**

It is with pleasure that I express my gratitude to Professor L. E. S. Eastham of the Zoology Department, Sheffield University, for providing me with research facilities in his department and for allowing me the use of the University bench at the Plymouth Laboratory. I am further indebted to Dr. H. R. Moon of the Zoology Department, University College, Leicester, for provision of facilities and assistance in obtaining material. I would
BLOOD CELL FORMATION IN CERTAIN TELOEST FISHES

like also to express my thanks to the members of the Staffs of the laboratories of the Marine Biological Association, Plymouth, and the Freshwater Biological Association, Wray Castle for their ready assistance and interest and to Professor D. Burns of the Physiology Department, King's College, Newcastle-upon-Tyne, for enabling me to complete the work.

REFERENCES

11 —, and —: Studies on lymphocytes. II. The origin and fate of lymphocytes in fishes. J. Morphol. 58: 529-546, 1924.
Blood Cell Formation in Certain Teleost Fishes

W. T. CATTON

Updated information and services can be found at:
http://www.bloodjournal.org/content/6/1/39.full.html

Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml