A CONTRIBUTION TO THE STUDY OF THE COMPARATIVE HISTOLOGY AND PHYSIOLOGY OF THE SPLEEN, WITH REFERENCE CHIEFLY TO ITS CELLULAR CONSTITUENTS

I. IN FISHES

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INTRODUCTION

A fairly extensive literature has grown up around the comparative histology and physiology of the spleen. The most comprehensive account is probably that of Whiting (89), who covers practically the entire vertebrate field, with the exclusion only of very low vertebrates such as the Cyclostomes. His account gives one an excellent general idea of the micro-anatomical structure of the spleen throughout the vertebrate series, but from the very vastness of the area covered it could not possibly be a really close and detailed study. Other observers have covered less ground with consequently greater thoroughness. Bannwarth (4), for example, made a very close study of the spleen of the cat. Similarly Mall (45, 46, 47) carried out a series of valuable researches on the spleen of the dog. A fairly detailed account of the literature as a whole may be found in the papers of Krumbhaar (32) and v. Skramlik (82).

As far as the spleen of Fishes is concerned, the most complete and systematic histological description is that of Phisalix (65), written in 1885. His account is not very satisfactory from a modern standpoint, in view of the considerable advances in knowledge and histological technique which have been made since then. He describes the cells of the blood as being either red or white, but makes no mention of what is now considered to be a fundamental subdivision of the white cells into granulocytes and non-granulocytes, not to mention the further grouping of the granulocytes in accordance with their affinities for acid and basic stains; Ehrlich's classical work on the various types of leucocyte had only been published a few years previously, in 1878 and 1879 (17, 18).

Many papers have been published concerning the anatomy and embryology of the spleen in various fishes, with numerous incidental observations on its histology (2, 12, 29, 33, 35–40, 43, 50, 52, 54, 57, 58, 59, 64, 67–70, 72, 73, 77). The most extensive work on the development of the spleen has been done by Laguesse, who also investigated in great detail the structure of the capsule and connective tissue framework in the spleen of Selachii (39, 40). Pouchet (72) in 1882

gave a good account of the "terminaisons vasculaires" or ellipsoids in the spleen of Selachii.

Concerning the physiology of the spleen in Fishes, both Whiting and Phisalix (*op. cit.*) make some references to it *en passant*. Phisalix (66) wrote in addition a brief note about the rôle of the spleen in the formation of red blood corpuscles in lower Vertebrates. Bizzozero and Torre (5) in 1883, investigating the process of erythrocytopoiesis in Fishes, make occasional references to the splenic cells, but like Phisalix they too seem to have known only one type of white cell. Drzewina and Pettit (16) investigated the physiological effects of splenectomy, and Pettit (62) noted the appearance of fusiform cells in the blood after removal of the spleen. Gueylard (23) and Miescher-Ruesch (53) investigated the changes occurring in the spleen of Fishes when transferred from fresh to salt water. Drzewina (15) in 1905 made a systematic histological study of the lymphoid tissue of Fishes, excluding the spleen; and to some of her findings reference will be made during the course of this paper. Hemmeter (26) in 1926 described the spleen of *Alopias vulpes*. Tait (85) in 1927 gave an interesting account of the spleen of the skate, with special reference to its vascular and reticular arrangements, and to an experimental investigation into the function of the splenic ellipsoids.

TECHNIQUE

The exclusive use of the routine histological method of examination, namely the preparation of sections (usually after embedding in paraffin), is open to very grave objection in an investigation of this kind. Apart from such factors such as shrinkage and distortion, a more serious source of error is introduced on account of the marked changes in staining reaction which may ensue. This is a grave defect in haematological work, especially where the Romanowsky stains are used, as with proper control the staining reaction with eosin constitutes the main criterion by which one usually decides whether a given cell contains haemoglobin or not (90). In this respect it is often difficult to know which step or steps in one's histological technique are responsible for certain tinctorial changes, so one can only judge any particular technique as a whole. After methyl alcohol, as used for example in Leishman's stain, the cytoplasm of both erythrocytes and granulocytes stains with eosin, but there is a very distinct and recognisable difference in tint between the two. After Zenker's fluid on the other hand this difference in staining reaction tends to disappear. In addition there is a further defect associated with the use of eosin. Not only does it stain cells which do not contain haemoglobin, but it often fails to stain those which do; young red cells, which on examination in the fresh condition may be definitely seen to contain haemoglobin, will nevertheless, as is well known, usually pick out the methylene blue in an eosin-methylene blue combination. Furthermore, as pointed out by Werzberg (88), the fixation technique itself may produce this inverted staining

reaction in the cytoplasm of the erythrocyte. Consequently, although the study of sections affords valuable information, it may be very misleading unless one's conclusions are checked by the examination of fresh and unstained cells. And even then a cell which contains only a small quantity of haemoglobin may be difficult to distinguish from one which contains none at all. Okajima's specific stain for haemoglobin (60), it may be mentioned, did not give good results.

Any work on splenic cytology which relies only on sections loses a large part of its value, for only in a fresh smear preparation can the various splenic cells be clearly identified. The ordinary splenic smear however is open to two objections. As a mechanical result of the act of smearing, portions of cell protoplasm may be drawn out into long processes, which may be very misleading if their origin is not clearly understood (fig. 1). Furthermore, the cells are scattered irregularly all over the slide, and their relationship to one another is thereby completely upset. In order to overcome these disadvantages the "anatomical smear" was devised. With a pair of forceps one takes hold of a piece of spleen, one end of which has been cut quite flat, and gently applies the cut end to a glass slide, instead of smearing it along the slide in the usual manner. In fact the preparation is really a stamp, not a smear. The result is that the cells are transferred to the slide in the same relative position to one another as they occur in the cut end of the organ, and one really has the equivalent, as far as the loose cells are concerned, of a section of the organ one cell layer in thickness (fig. 2). That at least is the ideal aimed at in the preparation. In actual practice however the anatomical smear is not uniform, for in some parts two or three layers of cells may be deposited on the slide, and only in parts is the ideal of one cell layer attained. But where this occurs the preparation is extremely valuable, for one not only sees the splenic cells uncut and undistorted, but also in their exact natural relationship to one another. It may be observed that two precautions are necessary in order to make a good anatomical smear. First, the organ must be drained of all excess blood, otherwise blood exudes from the cut surface and spoils the preparation. In the present work the removal of blood was effected as a matter of routine, for the animals were all killed by cutting off their heads and allowing the blood to drain away from the cut end, exsanguinating in this way the entire body, including the spleen. In the second place the cut end of organ must be perfectly flat, and to secure this a sharp scalpel, or better still an ordinary safety-razor blade, is necessary.

For an understanding of the spleen one must also have some knowledge of the functionally associated tissues. To understand what cells the spleen contributes to the blood, one must be acquainted with the cell content of normal blood, and with the other organs concerned in haemopoiesis. According to Giglio-Tos (21) haemopoietic "lymphoid" tissue occurs in the lamprey in the spiral valve of the intestine. In many Teleosts also, though not in any of the Elasmobranchs examined, haemopoiesis occurs in the

"lymphoid" tissue of the kidneys (fig. 3), and in other scattered "lymphoid" deposits of which Drzewina (15) has made a very careful study. The thymus also (80) may be concerned in blood formation.

The spleen was examined in paraffin sections, cut at 3–5 μ, after fixation in Helly's modification (25) of Zenker's fluid, which preserves the granules of the granulocytes. In many cases the spleen was washed out with normal saline until the returning fluid was perfectly clear, and then perfused with the fixative. The entire organ was then immersed in the fixative for one hour, after which it was cut into thin slices and fixation and subsequent treatment carried out in the usual way. This technique, as will be shown later, gives a beautiful demonstration of the pulp reticulum and the pulp spaces. Frozen sections of spleen were also made, after fixation in 10 per cent. formol (made with filtered fresh sea water), for the examination of fats and lipoids. Smear preparations were made of every spleen, and were first fixed in formol vapour for 10 seconds, the slide being held over a 40 per cent. solution of formalin at a distance of 1·0 cm., being then treated after exposure to the air for a few hours with Leishman's stain. Some form of preliminary vapour fixation is absolutely essential to obtain well-fixed preparations of blood films and splenic smears in Fishes. Werzberg (*loc. cit.*) and Rawitz (74) also mention the trouble they experienced in securing good fixation. Two or three films were made from the blood of every animal, and also sections of liver, kidney and swim-bladder occasionally, when the naked-eye appearances seemed to suggest haemopoiesis.

Frozen sections, having been made chiefly for the study of fats and lipoids, were stained with Scharlach R and Sudan III. Paraffin sections were stained with haematoxylin and eosin, haematoxylin and Van Gieson's picro-fuchsin, Mallory's aniline blue connective tissue stain, Heidenhain's iron-haematoxylin, occasionally with orcein for the demonstration of elastic tissue, and with a modification of Leishman's stain which gave some of the most instructive results of all. The section is grossly overstained for one and a half to two hours with the ordinary Leishman's stain diluted with an equal volume of distilled water, and then differentiated with methylated spirits (about 95 per cent. methyl alcohol) and absolute alcohol, followed by xylol and neutral balsam. This technique was originally devised in order that both the sections and the smears might be treated with exactly the same staining reagents (Leishman having first of all been found the best stain for the smears), and so any differences in the staining reactions of the various cells, on comparing the smear with the section, would be due to differences in fixation and other treatment, and not to the stain used. In this way one was enabled to compare the various techniques in so far as they affected the staining reactions of the cells and tissues. It was found however that this modified stain was so excellent in other ways that it was retained long after its original purpose had been subserved.

EXPERIMENTAL WORK

In addition to the material obtained from normal animals, a series of Fish were subjected to experimental procedures. This experimental work was confined purely to the common dogfish, *Scylliorhinus canicula*. As a result of observations made on the spleen of the normal dogfish, certain conclusions were arrived at concerning the mechanism of blood formation which it was desired to confirm by stimulating blood formation through the production of an experimental anaemia. This was done by withdrawing a known quantity of blood; after the lapse of varying intervals, the fish was killed, and its spleen and associated tissues examined in the manner already described.

There are in the dogfish no easily accessible superficial veins from which one can withdraw blood as in Mammals; but there are some superficial venous sinuses from which the withdrawal of blood seems at first sight an easy matter. These sinuses however are usually half empty, and the pressure of blood in them is very low. The systemic arterial blood pressure in the fish (i.e. in the post-branchial vessels) is only of the order of 7–10 mm. of mercury (6), owing to the fact that the blood does not pass from the heart to the systemic arteries direct, but must first of all traverse the branchial capillaries; as a result the pre-branchial pressure of about 45 mm. of mercury becomes greatly reduced. The venous pressure is almost invariably negative. Consequently it was found that the best and easiest method of obtaining blood was direct cardiac puncture, a procedure which is not followed by any ill effects, even when the heart is punctured ten or fifteen times.

The heart is reached in or very close to the mid-line, from the ventral surface, immediately anterior to the pectoral girdle. Actually in the mid-line itself the pectoral girdle has a small spur anteriorly, which can easily be felt through the skin, so that the needle can be inserted to the side of it. The needle is directed dorsally and caudally, and is best inserted first of all by itself, without the rest of the syringe. As soon as blood appears at the end of the needle, one knows that the heart has been reached, and the syringe is then attached. On the average 37 per cent. of the total blood was withdrawn, and in some cases over 50 per cent. The blood in Fishes constitutes only 2 per cent. of the total body weight (81) as opposed to 7–8 per cent. in Mammals. The procedure adopted was to weigh the fish immediately before the withdrawal of the blood, and withdraw where possible 1 c.c. of blood for every 100 gm. of body weight, thus giving a withdrawal of approximately 50 per cent. of the total blood volume. This would appear to be sufficient to stimulate fresh blood formation. (Exact details are given in the appended tables.)

Cardiac puncture cannot be performed unless the fish is first of all given a general anaesthetic. This is done very simply by placing the animal in a freshly made 2 per cent. solution of ether in sea water; the vessel containing the animal is covered over by a thick sheet of glass which is kept firmly

Table I

Some details of a series of 25 dogfishes in which experimental anaemia was produced. In each animal two sets of observations were made: (1) at the commencement of the experimental period, when blood was withdrawn by cardiac puncture; (2) at the close of the experimental period, when after a varying period, to allow for various stages in the degree of activity of the haemopoietic tissues, the animal was killed. In each case observations above are those at the commencement of the experiment, those below are at its termination. The first column gives the number of the fish, prefixed by the letters 2ES (Second Experimental Series), the second gives the times of commencement and termination of the experiment, the third the number of erythrocytes per cubic millimetre of blood at the two times, the fourth a similar count for the leucocytes, the fifth the amount of blood withdrawn (in c.c.), the sixth the weight of the fish in grammes, and the seventh the number and arrangement of the identification threads.

No. of fish	Duration of experiment	R.B.C.	W.B.C.	Amount of blood withdrawn c.c.	Weight of fish in gm.	Thread
2ES 1	12. vii. 28	123,000	15,000	1·5	425	W
	28. vii. 28				390	
2ES 2	12. vii. 28	105,000	17,500	3·5	450	B
	18. vii. 28					
2ES 3	13. vii. 28	193,000	20,000	5·5	870	WW
	10. viii. 28	151,000	23,000		820	
2ES 4	13. vii. 28	165,000	8,600	4·0	620	WB
	8. viii. 28	145,000	19,000		615	
2ES 5	13. vii. 28	159,000	42,000	2·5	660	BB
	13. viii. 28				690	
*2ES 6	14. vii. 28	333,000	45,300	6·0	620	BW
	3. viii. 28	229,000	24,000		575	
2ES 7	14. vii. 28	144,000	19,000	3·0	550	WWW
	31. vii. 28	155,000	23,000		620	
2ES 8	14. vii. 28	During the cardiac puncture the heart stopped beating altogether, and no blood was obtained. Recovered on return to tank				WWB
2ES 9	15. vii. 28	125,000	28,000	2·0	540	WBW
	6. viii. 28	124,000	13,500		565	
*2ES 10	15. vii. 28	176,000	39,000	6·5	800	WBB
	7. viii. 28	140,000	17,000		780	
*2ES 11	16. vii. 28	203,000	29,000	4·5	750	BBB
	13. viii. 28	141,000	9,300		730	
*2ES 12	16. vii. 28	270,000	39,000	6·0	740	BBW
	31. vii. 28	176,000	36,000		725	
2ES 13	16. vii. 28	181,000	42,000	7·5	745	BWB
	9. viii. 28	200,000	26,000		685	
*2ES 14	17. vii. 28	250,000	46,000	9·5	1075	BWW
	12. viii. 28	132,000	21,000		1085	
*2ES 15	17. vii. 28	306,000	45,000	7·5	865	WWWW
	6. viii. 28	103,000	14,000		885	
2ES 16	18. vii. 28	188,000	12,000	1·0 and 4·0	820	WWWB
	13. viii. 28	159,000	21,000		730	
2ES 17	18. vii. 28	151,000	9,900	2·0	460	WWBW
	26. vii. 28				440	
*2ES 18	19. vii. 28	249,000	41,000	6·5	840	WWBB
	30. vii. 28	165,000	25,500		810	
2ES 19	19. vii. 28			7·5	675	WBBB
	8. viii. 28	145,000	15,000		610	

TABLE I (*continued*)

No. of fish	Duration of experiment	R.B.C.	W.B.C.	Amount of blood withdrawn c.c.	Weight of fish in gm.	Thread
2ES 20	20. vii. 28	158,000	9,900	1·0 and 4·0	630	WBBW
	10. viii. 28	190,000	22,000		520	
*2 ES 21	21. vii. 28	226,000	40,000	6·0	685	WBWB
	12. viii. 28	114,000	29,000		665	
*2ES 22	21. vii. 28	204,000	33,000	8·0	895	WBWW
	26. vii. 28				810	
*2ES 23	22. vii. 28	241,000	31,000	6·0	630	BBBB
	13. viii. 28	139,000	20,000		580	
*2ES 24	23. vii. 28	220,000	45,000	7·0	690	BWWW
	8. viii. 28	144,000	29,000		615	
*2ES 25	25. vii. 28	183,000	31,000	8·5	875	BWWB
	13. viii. 28	120,000	15,000		880	

Nos. 1, 2, 5, 17 and 22 died in the tank; no blood counts were taken post-mortem.
Weight was gained by 5, 7, 14, 15 and 25.
Loss of weight was only slight in 4, 10, 11, 12, 17 and 21.

pressed down until the splashing ceases, and through which the progress of the anaesthesia can be watched. The first effect of the anaesthetic is a purely irritant one, the fish splashing violently and irregularly. As the ether begins to take effect, a true convulsive stage follows (corresponding presumably to the excitatory stage in human beings) towards the end of which the movements take the form of rhythmical side-to-side oscillation. Finally respiration ceases and the muscles become quite flaccid. The respiratory cessation however does not come on quite suddenly, but is preceded by a period of irregular and disorganised breathing, shown by twitching and tremor at the angles of the mouth. This tremor, it may be noted, is also the first sign of recovery from the anaesthetic. Anaesthesia is usually complete after five minutes—sometimes less, very rarely more. When anaesthesia is complete, the fish is taken out of the water, and after being weighed is placed upon a plain wooden board. Manipulative procedures may be carried out for about ten minutes, after which time as a rule twitching at the angles of the mouth shows that recovery is commencing, and the fish may then either be re-anaesthetised by placing it in the ether solution once more, or returned to its tank, where it will commence to breathe regularly again after about fifteen minutes. After twenty or thirty minutes it swims about apparently quite normally. Recovery may be expedited by the performance of artificial respiration, which is very simply done by drawing the fish to and fro in the tank, and so maintaining an artificial circulation of water through the gills. Although the anaesthesia thus induced lasts for about ten minutes, only three or four minutes are required as a rule for the actual withdrawal of blood and marking of the animal.

The marking of the fishes for subsequent identification offers several difficulties. The most practicable method was found to be by means of varying combinations of black and white silk threads, which were stitched on the fishes' backs immediately in front of the posterior dorsal fin. The threads are first of all soaked in paraffin wax, in order to make them more resistant to the action of the sea water, in which they may have to remain for several weeks.

One may here observe that the question which has so troubled anaesthetists whether, in death under anaesthesia, respiratory or circulatory failure comes first, admits of a very easy answer in the dogfish; the dissociation between the respiratory stoppage and the circulatory is very much longer than in higher Vertebrates, and the heart goes on beating for twenty minutes, and even more, after respiration has been completely suspended.

In order partly to confirm the account given by Tait(85) of the functions of the ellipsoids, and partly to see whether any light might be thrown on the mechanism by which lipoid particles are held in the wall of the ellipsoid, three dogfishes were given an injection of 10 c.c. of a 4 per cent. solution of Indian ink in sea water. In one case the injection was intra-muscular, and in the other two intra-peritoneal, and in each case the fish was killed three days after the injection. As in the previous experiments the fish were first of all anaesthetised.

The question of the precise relationship of the various Fish groups to one another in the evolutionary scale has not as yet been answered with certainty. The arrangement in this paper follows the account of Fish genealogy given by Kyle(34). Accordingly the Dipnoi will be described first, then the Elasmobranchii, and finally the Teleostomi, that being according to Kyle the order in which the main Fish groups have diverged from the ancestral tree. It is of interest to note that this arrangement had actually been decided upon to begin with, from examination of the spleens alone, before any reference had been made to descriptions of Fish biology.

THE SPLEEN IN THE DIPNOI. *CALAMOICHTHYS*

I have to thank Mr G. L. Purser, M.A., of Aberdeen University, for kindly lending me a piece of *Calamoichthys*' spleen. This unfortunately was the only specimen I was able to obtain of the spleen of the lung-fishes (their natural habitat being the tropics), but it is extremely interesting. It shows with almost diagrammatic clearness the essential elements of the spleen, and its circulatory mechanism, arranged in a more primitive manner than in any other spleen examined.

Running throughout the entire length of the organ are the artery and vein, placed side by side, and surrounded by a mass of "lymphoid" tissue. Surrounding the "lymphoid" tissue is the splenic pulp, covered by a thin capsule consisting of a single layer of cubical cells (fig. 4). The term "lym-

phoid," whose precise significance will be discussed later on, is throughout this paper used only provisionally.

When serial sections are examined it can be seen that at intervals the artery gives off branches which pass through the lymphoid tissue to enter the pulp; from the pulp the blood drains into the splenic vein, to reach which it has to pass a second time through the lymphoid tissue. The venules are much more numerous than the arterioles. The pulp consists of a cellular reticulum surrounding the lymphoid tissue; in the meshes of this reticulum is contained blood, and here also the splenic venules commence. It may be seen that the circulation is so designed that the blood flows (*a*) through the pulp reticulum, (*b*) around the lymphoid tissue. An important part of the splenic function is discharged by the formation in the lymphoid tissue of cells which enter the blood stream. Now there are only two ways in which this entry of lymphoid cells into the blood stream may be effected. In the first place they may pass through the walls of the arterioles and venules as they traverse the lymphoid tissue. There is however absolutely no histological evidence to support this, for the walls of the vessels as they pass through the lymphoid tissue, though thin, are quite complete; they show no signs of openings, nor anything to indicate the probability of openings being formed, to permit the passage of cells. The second and almost certain access is directly from the outer part of the lymphoid tissue to the blood which circulates around it in the pulp. The region therefore between the lymphoid tissue and the pulp is *the* site of cellular interchange between the lymphoid tissue and the blood, and will in future be referred to as the "boundary zone." In the boundary zone the blood as it passes through the splenic pulp sweeps away with it the outermost cells of the lymphoid tissue (fig. 4 *a*).

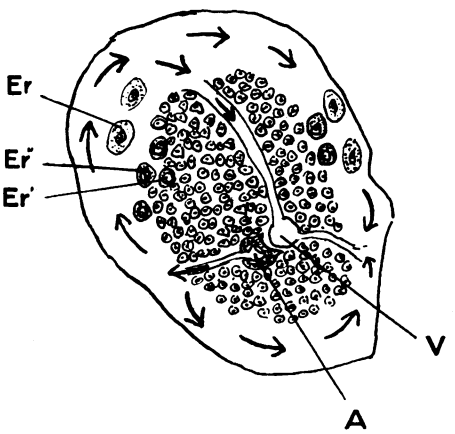


Fig. 4*a*.

It follows from what has already been said that in the boundary zone there is no sharp line of demarcation between the lymphoid tissue and the pulp; there is practically no connective tissue between the two, and the lymphoid tissue merges almost imperceptibly into the pulp, where the lymphoid cells may undergo certain phases of their development before entering the blood stream.

THE SPLEEN IN THE ELASMOBRANCHS

Of the cartilaginous Fishes there have been examined the common dogfish (*Scylliorhinus canicula*), the nursehound (*S. catulus*), the ray (*Raia clavata*), and the skate (*Raia batis*). For a detailed histological description the spleen of the dogfish has been chosen, for several reasons: it is the simplest to interpret, the dogfish is the most convenient animal for experimental work, and its cellular elements are large. The spleens of other fishes will be described only in so far as they differ from that of the dogfish.

SCYLLIORHINUS CANICULA

In contrast with the spleen of *Calamoichthys*, which has only two main elements—the lymphoid tissue and the pulp—the spleen of the dogfish has three essential constituents: (1) lymphoid tissue, (2) pulp, and (3) ellipsoids (fig. 5). The histological picture varies considerably according to the amount of blood which the organ contains. When the spleen is congested blood accumulates in the pulp, which consequently appears to be much more extensive than when the organ is first emptied of its blood, as happens when the fish is killed by cutting off its head and allowing all excess blood to drain away from the cut ends of the main vessels. Under such circumstances the pulp appears to diminish very considerably. What has happened is that the spongy reticulum, not having much blood in its meshes, undergoes almost total collapse. If however the spleen is perfused with normal saline, so as to wash out the last traces of blood, and then some fixative is run through, the pulp is prevented from collapsing, and its large-meshed spongy reticulum is beautifully shown (fig. 6). The capsule consists of a single layer of cubical cells with a thin stratum of connective tissue and a few elastic fibres underneath. Muscle elements are completely absent, and there is no trace whatever of a trabecular system.

The splenic arterioles terminate by dividing into three or four thick-walled capillaries, the ellipsoids, which then end by opening into the pulp spaces. The ellipsoids are really the splenic capillaries, differing from capillaries elsewhere merely in the greater thickness of their walls. The ellipsoids open directly into the spaces of the spongy pulp reticulum and commencing among the meshes of this reticulum are the splenic venules, which soon after their formation acquire a lymphoid sheath. Both arteries and veins therefore have a sheath of lymphoid tissue, as in *Calamoichthys*. Furthermore, it follows from what has just been said that the circulation through the splenic pulp is unquestionably an open one (figs. 6 and 7).

The ellipsoids practically never possess a complete lymphoid sheath, though often in contact with lymphoid tissue in part. They usually have a complete investment of pulp. Apart from the thickness of their walls the ellipsoids are also peculiar in that their lumen is usually very narrow, frequently to the verge of obliteration with a diameter of 5–6 μ, insufficient to

allow a red blood corpuscle to pass through without considerable compression and distortion. Hemmeter (26), examining the spleen of *Alopias vulpes*, appears not to have realised that the ellipsoids are blood vessels, but calls them "hemolytic islands." In contrast with the ellipsoids the lymphoid vessels have a larger lumen and permit of a much freer flow of blood.

Both the arteries and veins possess lymphoid sheaths, and so the communications of the lymphoid vessels vary accordingly. The arterioles lose their lymphoid sheaths and pass into the ellipsoids (fig. 8). The venules on the other hand, after their formation by the confluence of the pulp spaces, quickly acquire a coating of lymphoid tissue, and so this group of lymphoid vessels communicates directly with the pulp.

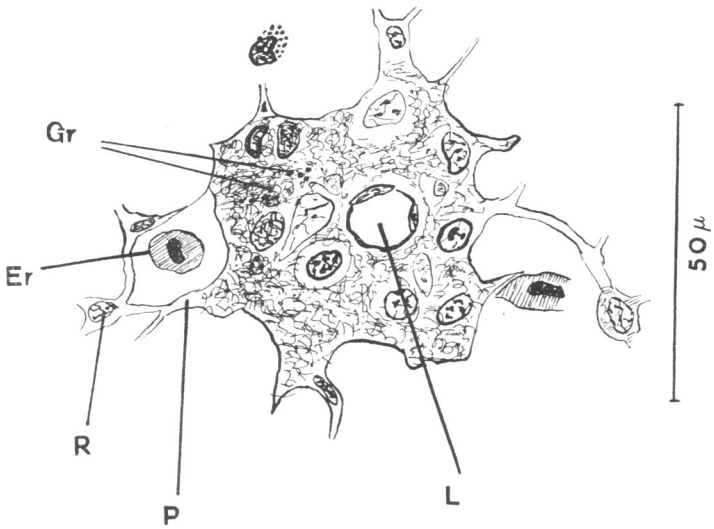


Fig. 9.

In an ordinary section (fig. 5) the wall of the ellipsoid is clearly and sharply defined, and on staining with Mallory or Van Gieson there is seen to be a condensation of connective tissue to form a thin but dense plate all round it. Nevertheless there are numerous processes extending from the wall of the ellipsoid into the pulp, where they become continuous with the processes of the pulp reticulum (figs. 7 and 9). These can be seen very clearly where the organ has been washed out with saline and then perfused with fixative.

The lymphoid tissue forms dense cellular areas which stain very deeply with nuclear stains. In the dogfish the lymphoid tissue is disposed in the form of a sheath around centrally placed blood vessels, and scattered among the lymphoid cells are large hyaline cells, with a cytoplasm which hardly stains at all with any of the stains used, and small pale often lobed nuclei. In general the term "lymphoid" appears to be used to denote any dense accumulation of small round cells, with relatively large nuclei and scanty

cytoplasm, bearing a superficial resemblance to the lymphoid tissue found in Mammals. Actually however there are very considerable differences between the lymphoid tissues of Fishes and Mammals, both in their component cells, and even more strikingly in the cells to which they give rise. In the dogfish for example the lymphoid tissue gives origin to all the cellular constituents of the blood, whereas in Mammals it has been believed until quite recently not to form any erythrocytes at all, and even of the white cells to produce only about one-third. In order however not to create any confusion by the introduction of new terms, the "lymphoid" tissue of Fishes will still be described as such, with the qualifications already mentioned.

Internally the lymphoid sheath is bounded by the wall of the vessel which is passing through it. The central lymphoid vessel varies in thickness according as to whether it is arterial or venous. The veins are very thin-walled. In paraffin sections, where there is very considerable tissue shrinkage, and the vessel wall appears in consequence to be even thinner than it really is, the impression is often produced that cells from the lymphoid tissue may enter the blood stream by passing through the thin vessel wall. In frozen sections however, where there is very much less shrinkage, it is obvious that the vessel wall is complete and uninterrupted, and that the small round cells do not enter the blood stream in this direct manner, but only obtain access to it indirectly by first of all entering the splenic pulp, and from there draining into the veins. It follows from this that externally the lymphoid tissue has no clear line of demarcation from the pulp; there is no connective tissue between the two, but the one merges imperceptibly into the other, so that it is impossible to say at what point the lymphoid tissue ends and the pulp begins.

The cells of the lymphoid tissue may be enumerated as follows:

1. *Small round cells.* These are the most numerous of all the cells to be found in the lymphoid sheath, and they stand out clearly by reason of their very scanty cytoplasm and the deep staining of their nuclei, which have well-marked limiting membranes and chromatin nodes. When seen in paraffin sections the small round cells vary very considerably in shape. In a splenic smear however they are all circular, and even more markedly than in the section they appear to consist almost entirely of nucleus, little or no peripheral cytoplasm being discernible. It is for this reason that French writers often refer to them not as cells, but as "Noyaux d'origine." In a smear preparation most of the nuclei are seen to be finely granular with a few larger chromatin nodules towards the centre. In those cells possessed of a cytoplasmic rim, the nucleus is usually finely reticulated.

2. *Large round cells.* These are about twice the size of the small round cells, and have more cytoplasm both relatively and absolutely. Their nuclei do not stain so deeply, the nuclear membrane, though well marked, is not so thick and dense, and the chromatin nodes are smaller and fewer than those of the small round cells. On the other hand a delicate intra-nuclear network is usually present. But although this description is applicable to a typical large round cell, there are so many intermediate forms between the large and the small round cell that there is no doubt the former arises by growth from the latter.

3. *Large hyaline cells.* These are very curious cells, and their occurrence is subject to very considerable variation not only in different spleens, but also in different parts of the same spleen. Owing to their large size they can be seen quite clearly under fairly low magnifications (×120) as clear rounded spaces, the cytoplasm taking up hardly any stain, with small central nuclei which are sometimes lobed, and stain as a rule very faintly (fig. 10). The hyaline cells are scattered irregularly throughout the lymphoid tissue.

4. *Cells undergoing karyo-kinesis.* These occur very infrequently in the normal spleen, and can only convincingly be demonstrated in those cases where the activity of the lymphoid tissue is experimentally stimulated. There is no collection of these mitotic cells to form a germ centre as described by Flemming in mammals (19). The dividing cells occur irregularly throughout the lymphoid tissue, tending to be rather more numerous near the central blood vessel, but not very markedly so.

5. *Granulocytes.* These occur mainly in the region of the boundary zone, in the outer portion of the lymphoid sheath. They are of two types, though in either case they are only the immature forms; for as soon as they approach maturity they leave the lymphoid tissue to enter the pulp and blood stream. One of the granulocytes I have ventured to call the "Perinucleate" cell, on account of the position of the nucleus, which is invariably situated at the periphery of the cell; and when the latter is seen in profile, the nucleus may be seen to be perched upon it. The nucleus is usually bilobed, but it may also be divided into three or four lobes, and so closely resemble the nucleus of the polymorpho-nuclear neutrophile leucocyte of higher vertebrates. The granules in its cytoplasm however are strongly eosinophilic. The perinucleate cell is about one and a half times the size of the small round cells, and its granules are spherical. In the second type of granulocyte on the other hand the granules are usually rod-shaped; in a smear preparation, more so in the ordinary than in the anatomical smear, the granules of both types of cell tend to be scattered all over the field. The nuclei of the neutrophiles are round or nearly so, never attaining the lobulation of the perinucleate nuclei, from which they also differ in being more centrally placed.

6. *Immature red cells.* Small round cells which are just commencing in the boundary zone to develop into erythrocytes, and then pass into the pulp.

Examination of the pulp in sections alone is very misleading. The length of an average red blood corpuscle is about 27 μ, and of the nucleus 10 μ, leaving about 8·5 μ of clear haemoglobin at either end of the cell. If through an erythrocyte of this size sections 3 μ in thickness are taken, there will be a maximum of ten sections altogether, only four of which will contain any of the nucleus, while in the other six sections portions of the haemoglobin-containing cytoplasm will be cut off without any nucleus. These will appear as seemingly isolated masses, which might be interpreted as fragments of haemoglobin from old broken-down erythrocytes, or alternatively as free haemoglobin accumulating for the formation of new erythrocytes; at any rate, whatever interpretation one were to put upon them, judging merely from the section one would come to the very paradoxical conclusion not only that there are present masses of free haemoglobin, but that they are nearly twice as numerous as the masses of nucleated haemoglobin, whereas actually they do not exist at all! It is therefore only in the smear that one gets any accurate conception of the true shape and appearance of the cellular constituents of

the spleen; and in a smear of the dogfish's spleen one sees at once that there are no fragments of free haemoglobin. With these preliminary remarks one may proceed to enumerate the cells of the pulp as follows:

1. *Reticulum cells.* The pulp proper consists of a loose cellular reticulum, in whose meshes are the free cells of the circulating blood. The spongy reticulum can collapse to a very small volume when emptied of blood, and on the other hand can open up considerably when the organ becomes congested, and so accommodate large quantities of blood. The reticulum establishes important connections with the smaller blood vessels. Processes of the reticulum cells are attached to the walls of the smaller splenic veins, which they help to strengthen. Laguesse(40) describes the reticulum cells as forming "cones of insertion" where they join the vein walls. This is not invariable, though quite frequent. The reticulum cells are also connected by numerous processes with the walls of the ellipsoids (fig. 9), as has already been mentioned. The cellular reticulum can only be clearly seen in those spleens which have been first of all washed out with normal saline to remove excess blood, and then perfused with the fixative. Any general stain will then serve to demonstrate the reticulum perfectly.

2. *Red blood corpuscles.* As seen in section these show extensive variation both in size and shape, but these variations are partly due to the well-known elasticity of the erythrocytes which allows them to be compressed into almost any shape, and partly to the fact that they are cut in different planes. Much more significant is the variation in the size and density of the nuclei of the red blood corpuscles. The nuclei of the adult erythrocytes in the general circulation are small and dense. In the splenic pulp however there also occur erythrocytes with nuclei which are larger and less dense in texture; between these and the mature erythrocyte with its small and more compact nucleus all types of intermediate cells are to be found, with nuclei diminishing in size and increasing in density until the appearance is reached of the mature nucleus of the circulating erythrocyte. Concurrently with the diminution in size of the nucleus, the haemoglobin content of the cell increases, so that there is roughly an inverse proportion between the two. When the cell is immature the nucleus is large and the haemoglobin scanty; as the cell becomes mature the nucleus becomes smaller and the haemoglobin more plentiful. Of especial interest is the fact that the younger erythrocytes, with large nuclei and scanty cytoplasm, are identical with the small round cells; and careful examination of the peripheral parts of the lymphoid sheaths reveals many small round cells which are just acquiring a narrow rim of haemoglobin. This process of the development of the small round cell into the erythrocyte can be seen to be going on even in the normal spleen, but the appearances are much more convincing and decisive in those cases where blood formation has been stimulated by the production of an experimental anaemia. Incidentally the fact that the nucleus of the adult erythrocyte is already a partially atrophic structure foreshadows the condition found in higher Vertebrates where the process of atrophy is carried to completion.

3. *Granulocytes, of both the eosinophile (perinucleate) and neutrophile variety.* The granulocytes occurring in the immediate vicinity of the lymphoid sheath, i.e. the boundary zone, are immature. Even more obviously than the young red cells, they are being formed from the small round cells by the acquisition of a narrow granular rim which gradually increases. At the same time the nucleus, at first perfectly spherical, becomes more irregular in shape and finally attains the lobulated condition which characterises the mature granulocyte (fig. 11, 1–13, and fig. 12).

The ellipsoids in paraffin sections present a finely granular and reticular appearance, the reticulation only showing up under high magnification

(× 600 +). In the meshes of this reticulum small granules of brownish pigment may be seen occasionally, and now and again a red blood corpuscle. The presence of erythrocytes in the walls of the ellipsoids is shown much more clearly in the spleen of the ray, and also in many Teleosts. There are several nuclei in the ellipsoidal wall, and as definite cell boundaries are usually absent, it would appear to be syncytial in nature. The red blood corpuscles in the ellipsoidal wall are obviously undergoing haemolysis, and it was this no doubt which led Hemmeter(26) to describe the ellipsoids as "hemolytic islands" without perceiving their relation to the blood vessels.

A fact which has not been mentioned by previous observers is the frequent appearance in the ellipsoid wall of considerable quantities of lipoid granules. In a frozen section of dogfish's spleen it can be seen that these granules accumulate mostly in the outer part of the ellipsoid wall, and here and there they may be seen passing into the pulp (fig. 13). This fact would appear to be of considerable significance in connection with the physiology of the ellipsoid.

The physiology of the dogfish's spleen.

From the histological appearances which have just been described it is possible to arrive at certain conclusions concerning the physiology of the dogfish's spleen. Blood entering the spleen passes through lymphoid tissue, through an ellipsoid, and so enters the pulp (figs. 7 and 8). During its passage through the ellipsoid it is exposed to considerable resistance owing to the great narrowing of the lumen, and in consequence of the increased resistance its rate of flow is very much reduced. Presumably the more fragile corpuscles become broken down, and those which are not fragile already tend to become so. The red cells as a whole, or the fragments resulting from their disintegration, are ingested by the phagocytic cells in the wall of the ellipsoid, where they undergo complete breakdown. Presumably their degeneration products are in part the source of the pigment granules which one sees in the ellipsoid wall, and in part, though this cannot be seen, pass into the pulp where they are employed in the formation of fresh red cells. It is highly probable that the breaking down erythrocytes in the ellipsoidal wall undergo some fatty change; this would account for the presence of the lipoid particles. Certainly it seems more feasible to assume that the breakdown of the erythrocyte is the source of this lipoid accumulation, than to assign to it some function altogether apart from the haemopoietic system, in connection with the general metabolism of fats and lipoids.

Tait(85) in some recent experiments showed that Indian ink particles injected into the blood stream rapidly disappeared from the circulation, and were then to be found in large quantities in the walls of the ellipsoids. This finding of Tait's was confirmed in the present experimental work, details of whose technique have already been given. "In fishes just as in mammals the sessile phagocytic cells of the ellipsoids capture foreign particles introduced into the blood stream, and probably also ingest effete corpuscular

elements of the blood" (Tait). Tait only observed with certainty the ingestion of Indian ink particles. It is in addition undoubtedly true that in most Fishes the ellipsoidal phagocytes ingest corpuscular elements in the form of erythrocytes, but not granulocytes.

During the passage of the blood through the pulp, it comes into contact with the outer part of the lymphoid sheath, and in this boundary zone cellular interchanges take place. Between the pulp and the lymphoid sheath there is practically complete absence of connective tissue, so that the cells can move about quite freely. The mere fact that connective tissue is so completely lacking at the boundary between two such cellular tissues as the pulp and the lymphoid sheath would in itself, merely from the structural point of view and quite apart from all other considerations, strongly suggest that there are occurring cell migrations with which the presence of connective tissue would interfere. From the lymphoid tissue cells are continually entering the pulp, from which they pass into the general circulation. The small and large round cells may either enter the blood as such, and then presumably circulate unchanged, although it is quite possible that even after they have entered the blood they may undergo further differentiation; or they may remain for a while *in situ*, and undergo the first stages of their development into erythrocytes or granulocytes, entering the blood stream to complete only the final phases of their development. The lymphoid tissue therefore gives rise to all the cellular elements of the blood, and is the fundamental haemopoietic tissue. In the ellipsoids on the other hand there would seem to be a mechanism for blood destruction.

A point of interest in connection with the maturation of the erythrocytes in the dogfish is that a large part of it occurs in the blood stream itself. In the blood of a normal dogfish one may constantly see many immature red cells. They can easily be picked out by reason of the fact that their cytoplasm contains less haemoglobin than the mature cell, and their nucleus is larger and much less dense. They become much more numerous when erythrocytopoiesis becomes more active, as in the present experimental series.

RAIA CLAVATA AND *RAIA BATIS*

Both the common or thornback ray (*Raia clavata*) and the skate (*Raia batis*) belong to the same fish group as the dogfish, and the essential features of their spleens are very similar. The capsule is rather thicker, and consists of several layers of fibrous tissue covered by a thin layer of flattened epithelium. The ellipsoids are thinner than those of the dogfish, having an average diameter of about 60 μ. They show even more clearly than in the dogfish a definitely reticulated and granular appearance, and the presence of red blood corpuscles in their wall, in varying degrees of dissolution, may be very frequently observed (fig. 14). The pulp is more extensive than in the dogfish, and its cellular reticulum is more dense. Tait (*loc. cit.*) observes that

"the lymphoid tissue is not an essential element in the structure of the spleen." This is a statement with which the histological findings in the present work are not in agreement. Lymphoid tissue is of universal occurrence in all the spleens examined, and is as "essential" an element in the structure of the spleen as any other, if it is not in fact the most essential in the spleen of Fishes, from the point of view of haemopoiesis.

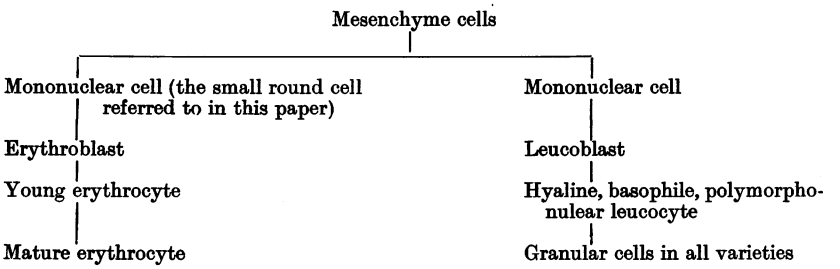
The cells in the spleens of the ray and skate are, broadly speaking, the same as those of the dogfish. The eosinophile leucocyte has very large granules which are only loosely attached to the cell, and which are set free in large numbers when one makes a smear or a blood film; one often sees nuclei with only a few granules adherent, the majority having become detached from the cell. In fact one almost receives the impression that the cell itself actively discharges its granules, in a more or less explosive manner. In the neutrophile leucocytes the granules, as in the dogfish, are distinctly rod-shaped. The erythrocyte is formed from the small round cell as in the dogfish, but its nucleus undergoes very little shrinkage or condensation during the process of its development, and so the nucleus of even the adult red blood cell is almost identical with that of the parent small round cell. One can also see all the stages intermediate between the small round cell and the fully developed granulocyte. Giant erythrocytes occur, and possess many points of resemblance to the megaloblasts of human blood. They are about twice the size of the ordinary erythrocyte, but have a relatively much larger nucleus which is markedly reticular, while their cytoplasm is poor in haemoglobin. They are not red cells about to divide, as no intermediate forms occur of direct or indirect division. They appear at first sight to be erythrocytes which are developing from large instead of from small round cells. Even this is doubtful, however, and probably they are normal intermediate forms in erythrocyte formation, for reasons shortly to be mentioned.

BLOOD FORMATION

A full account of the literature concerning the morphology and development of the blood in Fishes is given by Dean (13). Giglio-Tos (21) in 1896 described blood formation in the lamprey, and showed that it was localised in the spiral valve of the intestine. Bryce (7, 8) in 1905 gave an interesting account of haemopoiesis in the larva of *Lepidosiren paradoxa*; the conclusions arrived at in this paper agree almost exactly with his. Weidenreich (87) in 1905 gave an excellent critical summary of all the previous work. Rawitz (74) in 1899 and Werzberg (88) in 1911 also made extensive investigations into the morphology of Fishes' blood. Werzberg's paper in particular is very valuable, as he examined a large number of Fishes.

It is clear from the accounts already given that as far as Elasmobranchs are concerned (and the same applies to Teleosts also) the evidence is definitely against the polyphyletic theory of blood formation, and in favour of the

monophyletic theory. In the Elasmobranchs this fact stands out with extraordinary clearness. The common ancestor of the various blood cells would appear to be a small round cell, consisting mainly of nucleus, with little or no cytoplasm. It is by reason of the initial scantiness of the cytoplasm that differentiation into such widely divergent cell types as the erythrocyte and the granulocyte becomes possible, such differentiation depending primarily upon the kind of cytoplasm which this small round cell acquires, and secondarily upon changes in the nucleus following the acquisition of this cytoplasm. The scheme given by Bryce(8) to illustrate the second or post-larval phase of haemopoiesis in *Lepidosiren paradoxa* applies almost equally well to blood formation in the adult Elasmobranch:



The first step in the evolution of the small round cell into the erythrocyte is a slight swelling of the nucleus and the acquisition of a narrow rim of cyto plasm, which at first contains little or no haemoglobin. This cytoplasmic rim is usually homogeneous in its early stages; as it grows the haemoglobin content progressively increases. At the same time small rod-shaped actively motile bodies make their appearance in the cytoplasm. In a blood film prepared in the ordinary manner they do not stain, but they stand out very clearly when stained by a "vital" stain such as brilliant cresyl blue or neutral red. They do not stain with janus green B. They are not present in the very young red cell; they disappear almost completely in the adult red cell. In the intermediate stages they are very numerous and conspicuous. Giglio-Tos described similar "granulations" in the lamprey, and Sabrazes and Muratet (78, 79) in some other fishes. Giglio-Tos's view that they represent haemoglobin-forming substances is highly probable, for by the time the erythrocyte has acquired its full complement of haemoglobin they have disappeared. The acquisition of haemoglobin only proceeds up to a certain stage, at which the erythrocyte is said to be mature. At the same time as the cytoplasm is increasing the nucleus also enlarges, and becomes reticular, but with further increase in the cytoplasm the nucleus undergoes retrogressive changes, becoming shrunken and condensed.

What exactly are the limiting factors which allow the growth of the cell to proceed so far and no further it is difficult to say. The blood cells are different from the cells of most other tissues in that once they enter the blood stream they cease to be under the control of the central nervous system;

and they are also free from another growth-limiting factor, the pressure of adjacent cells. Therefore one must assume one of two things. Either there is some chemical agent present in the circulating blood which prevents the further growth of the cells once they have entered it. For this some of the recent work on tissue culture seems to hold some justification (3, 9, 10, 11). Or else the check may come not from without, but from within the cell itself; the specialisation of the cytoplasm has a nocuous effect upon the nucleus, and so upon the cell as a whole, so that it undergoes a degenerative process sufficient to prevent it from multiplying, but not to cause its complete breakdown. The fact that the nucleus of the mature cell is atrophic would fit in equally well with either hypothesis, the only question being one of *post hoc* or *propter hoc.*

The development of the erythrocyte from a non-haemoglobiniferous small round cell has been described by numerous observers (29, 42, 56, 88). Bizzozero (5) maintains that every erythrocyte is only formed by the division of a pre-existing erythrocyte, while Jolly (30), agreeing with Bizzozero that this mode of erythropoiesis occurs, at the same time does not exclude its formation from the small round cell. As far as the present work is concerned all one can say is that the erythrocyte in the vast majority of cases develops directly from the small round cell. The mature erythrocyte is quite definitely incapable of division, for when pyknosis occurs further nuclear activity is out of the question. It is, on the other hand, more than probable that the immature erythrocyte is capable of division. It is often very difficult to decide whether these dividing cells contain haemoglobin or not; if they do it can only be in small quantity, and not in the amount present in the mature cell.

Bizzozero's great objection to the development of the erythrocyte from the small round cell is that he does not believe a non-haemoglobiniferous cell can ever acquire haemoglobin. The obvious answer is that the first erythrocytes which come into existence certainly acquire their haemoglobin somehow, so that the development of haemoglobin in a cell which did not previously contain it, far from being impossible, does actually occur at one stage of development; and there is no reason why it should not occur again. Jolly, while believing that the erythrocyte can divide to form fresh red cells, asserts that before it does so it first of all loses its haemoglobin, and the daughter cells formed by its division have to reacquire their haemoglobin *de novo.* Thus, while agreeing with Bizzozero in the power of the red cells to divide, he disagrees with Bizzozero's main objection to the other method of erythropoiesis, namely from a non-haemoglobiniferous cell. Jolly's main work, it may further be noted, was carried out on amphibian blood, not fishes', and it is possible that there may be considerable difference between the two.

The first step in the development of the granulocyte from the small round cell resembles very closely the first step in the formation of the erythrocyte, and consists in the acquisition of a narrow rim of granular cytoplasm; the granules at first are not as large, nor do they stain so deeply, as those of the

adult cell. In the early stages of their growth the nuclei of the erythrocyte and the granulocyte are exactly the same. As a result of nuclear changes however the two cell groups diverge still further from the parent type. In the case of the erythrocyte the nuclear change is one of shrinkage and condensation. In the case of the granulocyte there is only slight condensation, but together with it there occurs lobulation, so that the nucleus may ultimately form two or even three distinct lobes, as in the adult perinucleate cell. Drzewina (15) had some idea of this possible development of the granular cell from the lymphocyte, observing in the lymphoid tissue of the sturgeon's kidney a whole series of intermediate forms between the two types of cell, but she did not come to any definite conclusion. In the present work these various transition stages have been met with so frequently, in all the Elasmobranch spleens examined, that there is no room for doubt in the matter. Lowitt (42) believed that there were two different types of small round cell, from one of which the erythrocyte developed, from the other the granulocyte. In the Elasmobranch spleens, more particularly in those of the ray and skate, there seems at first sight to be some evidence of this being so. But careful examination shows that what actually happens is that the nucleus of the small round cell begins to undergo slight changes very early on, before it has acquired any obvious coating of granules or haemoglobin.

The method used in the present work for the production of anaemia has already been described. Jolly (30), using *Triton cristatus*, produced anaemia by starving the animal for three to five months and then feeding abundantly with "red worms." This procedure results in widespread mitosis of all the body cells, including the red blood corpuscles. One can hardly doubt the accuracy of Jolly's findings, but at the same time it seems clear that prolonged starvation induces a totally abnormal state of affairs, and one must exercise great caution in making deductions therefrom as to the normal method of blood formation. Starvation for example would create a shortage of haemoglobin, in virtue of which it seems more than likely that none of the erythrocytes would ever become fully mature, and in consequence the final nuclear changes of shrinkage and condensation would not supervene. It is therefore highly probable that the dividing erythrocytes observed by Jolly were all immature forms. Clearly the immature erythrocyte is much more likely to be capable of division than the mature erythrocyte. For the small round cell can undoubtedly multiply, and the young erythrocyte, before degenerative changes have occurred in its nucleus, is after all only a small round cell with a little cytoplasm added.

Laguesse (36), working with trout embryos, produced anaemia by cutting off their tails. Bizzozero and Torre (5), working with *Carassius auratus*, bled the animals four times in fourteen days by cutting on each occasion one of the branchial arteries. In both these instances it is very difficult to gauge the degree of anaemia produced. In the present work, on the other hand, the degree of anaemia could be estimated with considerable accuracy, and as

may be seen from the appended list, 37 per cent. of the total blood was withdrawn on the average, and in some cases 50 per cent. or over.

In Bizzozero's experiments fresh blood formation (in the form of mitosis of erythrocytes) was not observed until sixteen days after the last bleeding, or thirty days after the first. In the present work the animals were examined at intervals varying from six to thirty-one days after the withdrawal of blood. New blood formation begins to be evident by about the sixth day, reaching a maximum about the twentieth, as indicated by the appearance in increased numbers of immature cells both in the blood and the spleen. The only undoubted method of blood formation observed was that which has already been described, namely from the small round cell. No mitoses of granulocytes were observed throughout the entire series; occasionally mitoses were seen which might have been either small round cells or immature erythrocytes.

THE SPLEEN IN THE TELEOSTS

Of the Teleosts the following members have been examined: *Pleuronectes platessa* (plaice), *P. flesus* (flounder), *P. limanda* (lemon-dab), *P. microcephalus* (common dab), *Gadus merlangus* (whiting), *G. minutus* (poor cod), *G. luscus* (whiting pout), *G. pollachius* (pollack), *G. morrhuae* (common cod), *Lophius piscatorius* (angler-fish), *Trigla gurnardus* (grey gurnard), *Morone labrax* (salmon bass), *Molva molva* (ling), *Spinachia vulgaris* (fifteen-spined stickleback), *Callionymus lyra* (dragonet).

Generally speaking, the Teleosts are a much larger and more heterogeneous group than the Elasmobranchs. The three main splenic elements, lymphoid tissue pulp and ellipsoids, are present in all except the dace, where the ellipsoids seem to be almost completely absent.

The capsule is like that of Elasmobranch spleens, except that in many Teleosts there are often to be found embedded in it varying amounts of pancreatic tissue. These outlying fragments of pancreas sometimes follow the track of the splenic vessels into the interior of the spleen. This is well marked in the *Pleuronectes* and *Gadus* families, and best of all in *Molva molva*. The pancreatic elements are always separated off from the splenic substance proper by a layer of fibrous tissue. As in Elasmobranchs so in the Teleosts there is no trace whatever of muscle in the capsule, or of a trabecular system. In *Trigla gurnardus* there are a few delicate septa of fibrous tissue passing from the capsule for a very short distance into the splenic substance.

The ellipsoids show some interesting variations in different species. In *Lophius piscatorius* the reticular meshwork in the ellipsoidal wall stands out very clearly, while surrounding the ellipsoids are large sinus-like spaces. These spaces only occasionally contain blood, and then in small quantities; it would appear that they are occupied mainly by lymph. They have a definite endothelial lining and are traversed by fine strands of connective tissue passing between the ellipsoid and the outer wall of the sinus. Most Teleosts show very

clearly the presence of disintegrating erythrocytes in the ellipsoidal walls. The ellipsoids attain a particularly high degree of development in *Molva molva*. Here they are often eccentric in shape, and have numerous gaps in their walls. In a Mallory stained section it may be seen that the condensed sheet of connective tissue which surrounds the ellipsoid becomes thinned out and deficient opposite these gaps, through which blood seems to pass to enter the pulp, and the main axial lumen of the ellipsoid often seems to end blindly. Pouchet (72) has described this method of ellipsoidal termination as occurring in Elasmobranchs, but this I have been unable to confirm.

Lymphoid tissue tends to be more diffuse in Teleosts than in the Elasmobranchs. It is very well developed in *Molva molva*, and tends to form definite nodules in *Morone labrax*. In most Teleosts however lymphoid tissue seems very diffuse, sometimes almost inextricably intermingled with the pulp. One very distinctive feature is the development in Teleostean lymphoid tissue of pigment nodules. These were present in all the spleens examined, with the exception of *Spinachia vulgaris*. In addition to their association with lymphoid tissue, the pigment nodes sometimes (e.g. in the flatfishes) have definite vascular relationships, surrounding the smaller arteries and veins. They also occur in the lymphoid deposits found in other parts of the body besides the spleen, e.g. the lymphoid tissue of the kidney (fig. 3). They have a fairly sharply circumscribed outline as a rule, though there is usually no definite connective tissue layer surrounding them to account for this appearance. As a rule they are paler towards the centre, and darker towards the periphery. Their chief contents are small spherical masses of brown or greenish brown pigment, which may often be observed to consist of small closely packed brownish granules. Occasionally one receives the impression that these masses are pigment-containing cells, as there may be a suspicion of a nucleus present; usually however this is not the case. In addition there are frequently, though not always, to be found in the pigment nodules erythrocytes and small round cells in the process of disintegration. The pigment nodes are rich in lipoids in their central clear portion. Histo-chemical tests for iron also show that they have a high iron content. The pigment is quite evident even in unstained sections, but curiously enough one sees very little of it in splenic smears. The colour of the pigment is strongly suggestive of bile, a view which is favoured by the frequent occurrence of disintegrating red cells. Similar pigment masses, more numerous but smaller in size, are to be found in the dogfish's liver.

The details of haemopoiesis were much more difficult to follow in Teleosts than in Elasmobranchs. In those cases where it was possible to come to any conclusion, the usual mode of erythropoiesis was seen to occur. The gurnard (*Trigla gurnardus*) shows two interesting phenomena in connection with the development of the red corpuscles. The parent small round cell has in its cytoplasm large basophile granules. As the cell enlarges and acquires its coating of haemoglobin, these granules become smaller and fewer. Just before

the final stages of erythrocyte formation, the cell cytoplasm consists of small scattered bluish granules on a dull pink background. The appearance resembles very strikingly the condition of punctate basophilia found in human blood in Addisonian anaemia, although of course it is not suggested that there is any analogy between the two, or that the occurrence of punctate basophilia in human beings indicates a reversion to a piscine ancestry.

In the *Gadus* group the erythrocytes may assume a very curious shape. At first round, they then become oval, as in other fishes. They then show an increasing tendency towards angularity, and finally may become perfectly triangular in shape (fig. 15). The relative proportion of triangular to oval red blood corpuscles varies from one animal to another. The illustration shown is from a blood film of *Gadus minutus* in which the majority of the erythrocytes are triangular. On the other hand there are many cases in which only a few of the corpuscles are triangular, and the majority are of the normal shape. The triangularity is not artificially produced by the fixative because it may be observed in specimens of perfectly fresh and unfixed blood, though the angles may not be sharp as in the fixed film.

Another interesting variation which may occur is connected with the nucleus of the erythrocyte. It has already been observed that in the course of development of the small round cell into the erythrocyte or the granulocyte, there is in each case a certain amount of nuclear degeneration, such degeneration in the case of the erythrocyte taking the form of shrinkage and condensation, whereas in the case of the granulocyte the nucleus becomes lobulated. In the erythrocytes of the *Pleuronectes* group quite frequently, and in other Teleosts occasionally, the nuclei of the erythrocytes also may be lobulated.

The granulocytes in Teleosts are not very well marked. Eosinophiles in particular are sometimes very inconspicuous, so much so that Sabrasez and Muratet (quoted from Bashford Dean's bibliography) believed them to be absent altogether from Teleost blood. This however is not the case. In the *Gadus* family they are quite numerous and easily found. But it is certainly true that they are not as uniformly plentiful or as intensely eosinophilic as they are in Elasmobranchs. In *Trigla gurnardus* the neutrophile leucocyte possesses both basophile and eosinophile granules, the basophile surrounding the nucleus, and the eosinophiles occupying the periphery of the cell. The cell would more correctly be called ambophile. A similar phenomenon has been noted by Drzewina in other fishes, and she questions the orthodox view as to the specificity of the granulocytes, at any rate as far as lower Vertebrates are concerned. It is quite common in most Fishes to note that the pinkish granules of the neutrophiles lie against a faint blue background, but the blue-staining substance is not granular.

In the blood of the gurnard (*Trigla gurnardus*) there occur giant erythrocytes, and their occurrence raises the very important question "What is the mechanism which ensures that the growth of the erythrocytes shall only

proceed up to a certain point?" With this is associated the question as to why all the erythrocytes should be of the same size. In addition to what has been observed previously, in considering why cells circulating freely in a fluid medium should cease to grow at all, there is an important mechanical factor to be taken into consideration, and that is the bore of the capillary through which the erythrocytes have to pass. If the diameter of the erythrocyte is greater than that of the capillaries, it will undergo compression each time it passes through, causing after frequent repetition its elongation in the line of the long axis of the capillary, and its shortening in the direction of its compression, that is transversely to the line of blood flow. In this way, by compression in one axis and elongation in the other, one may possibly have an explanation for the change in shape of the erythrocyte, from the spherical when it is young and attached to the lymphoid tissue, to the oval as it matures and enters the general circulation, where it is subjected to the moulding action of the capillaries. One might of course argue in the opposite direction, that the bore of the capillaries is determined by the size of the erythrocytes which force their way through. But that could only apply very early on in development when the capillaries are first being formed. Once the capillaries are fully formed and their mean calibre fixed, one would suppose that the blood cells have little, if any, further action upon them, whereas their influence upon the blood cells will continue throughout life, as long as blood is being formed. If for any reason (such as the parent cell being larger than usual or possessed of greater developmental activity) the erythrocyte grows more than the normal, it can easily grow to twice its length and still be able to traverse the capillaries, but it cannot grow in width with quite the same facility. Hence one would expect giant erythrocytes to be only a little wider than normal, but considerably longer; and this is what actually happens. In the blood of the gurnard the average erythrocyte measures $6\mu \times 8\mu$, and the giant variety $7{\cdot}5\mu \times 14\mu$.

The nuclear degeneration in the erythrocyte of Fishes does not usually proceed beyond the stage of shrinkage and condensation. In exceptional cases however this state may be followed by one of chromatolysis, the nuclear substance then mixing with the remaining part of the cell to a greater or lesser degree. In Mammals of course this mixture of nuclear substance is normally complete, giving rise to the homogeneous mass which constitutes the mammalian red blood corpuscle. It is important however to note that this is not an absolutely new phase in the life cycle of the erythrocyte, developing exclusively in Mammals, but on the contrary that indications of it may be seen in the blood of even such primitive Vertebrates as Fishes.

SUMMARY AND CONCLUSIONS

1. The present paper is based upon a detailed examination of the spleen, blood, and kidneys of a number of Fishes.

2. The Fishes examined are *Calamoichthys, Scylliorhinus canicula, Scylliorhinus catulus, Raia clavata* and *Raia batis, Pleuronectes platessa, P. flesus, P. limanda* and *P. microcephalus, Gadus merlangus, G. morrhua, G. luscus, G. pollachius* and *G. minutus, Lophius piscatorius, Trigla gurnardus, Morone labrax, Molva molva, Spinachia vulgaris, Callionymus lyra,* and *Leuciscus leuciscus.*

3. A modification is described of the ordinary technique for making smear preparations of the spleen or any other cellular tissue. There is also described a method for the application of Leishman's stain to paraffin sections.

4. In the Dipnoi (Lung Fishes) there exists a very primitive type of splenic circulation, arranged so that the blood may flow around the lymphoid tissue and receive from it the developing blood cells.

5. In the Elasmobranchs (Cartilaginous Fishes) there is to be observed an essentially similar arrangement, the splenic circulation being so designed as to enable the blood to circulate through the pulp and around the lymphoid tissue.

6. The same may be said of the Teleosts, but on the whole the arrangement of the splenic elements is much less precise in the Teleosts than in either the Dipnoi or the Elasmobranchs.

7. In Elasmobranchs, most markedly in the dogfish, the ellipsoids contain in their walls lipoid granules; in paraffin sections where these granules do not show, there is to be seen in the wall of the ellipsoid a fine reticular formation.

8. In most Fishes (e.g. the ray, plaice and ling) there are present in the wall of the ellipsoid red blood corpuscles in various stages of disintegration. It is inferred from this that the ellipsoids play an important part in the mechanism of blood destruction. The ellipsoids also help in the removal of foreign particles from the blood stream.

9. The capsule in all the fishes examined is very thin, and consists of a single layer of epithelium with a thin stratum of fibrous tissue underneath. Muscular tissue is not present, and there are only a few elastic fibres. There is no indication whatever of a trabecular system.

10. The pulp consists of a spongy cellular reticulum through which the blood percolates.

11. In all the Elasmobranchs examined, and in the vast majority of the Teleosts, the arteries terminate by dividing into three or four short thick-walled capillaries, the ellipsoids, which open directly in the spaces of the pulp reticulum. The veins commence by the confluence of these pulp spaces, so that the circulation through the spleen is unquestionably open. There are no ellipsoids in the Dipnoi, if one may generalise from the single specimen examined, but there also the circulation seems to be an open one.

12. The region where pulp and lymphoid tissue adjoin one another has been called the boundary zone; in the boundary zone there is no connective tissue barrier between the pulp and the lymphoid tissue, and cellular interchanges occur in this region quite freely between the lymphoid tissue and the pulp.

13. The lymphoid tissue of Fishes gives rise to all the cellular elements of the blood. In Teleosts it is also the seat of certain pigmentary changes whose precise significance is obscure. Although the term "lymphoid" is retained in this paper, the tissue so named is very different from the lymphoid tissue of Mammals.

14. The lymphoid tissue of Fishes is composed mainly of aggregations of small round cells. These cells have a relatively large spherical nucleus, with little or no cytoplasm.

15. The original small round cells may develop into either erythrocytes or granulocytes. This process may be best seen in the Elasmobranchs.

16. In order to demonstrate the phenomena of haemopoiesis more clearly, blood formation was stimulated in a number of dogfishes by the experimental production of anaemia. Full details of the technique and the results obtained are given in the text.

17. The blood cells in Elasmobranchs, and in all those Teleosts where it was possible to form a definite opinion, are derived from one common parent cell.

18. The occurrence of abnormally large erythrocytes leads to a consideration of the factors which control the growth of the blood cells. No satisfactory conclusions are reached.

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DESCRIPTION OF FIGURES AND ILLUSTRATIONS

Fig. 1. Smear of dogfish's spleen (× 60) prepared in the ordinary manner. Note the confused arrangement of the cells and the artificial cell processes.

Fig. 2. Smear of dogfish's spleen, made according to the technique described for the anatomical smear. Note the absence of cell processes. S = the lighter coloured area in the middle of the field corresponding to the lumen of a blood vessel. It is surrounded by more closely packed small round cells.

Fig. 3. Kidney of *Pleuronectes platessa* (plaice) (× 60). T = renal tubules cut in transverse section. L = an irregular mass of lymphoid tissue in between two tubules. P = a pigment node, showing in the photograph as a black area in the midst of the greyish lymphoid tissue. There are no glomeruli to be seen in the section; they seem to be much more infrequent in the kidney of Fishes than in higher Vertebrates.

Fig. 4. Spleen of *Calamoichthys* (× 60). A = the thick-walled splenic artery, with blood in its lumen. V = the thin-walled splenic vein, whose lumen is empty. v = a tributary of the splenic vein, arising in the pulp and coursing through the sheath of lymphoid tissue in which the main artery and vein are situated, somewhat eccentrically.

Fig. 4 *a*. Schema, based upon fig. 4, to illustrate the essentials of the splenic circulation in *Calamoichthys*. The central artery and vein are surrounded by lymphoid tissue, and outside this is the pulp, which, for the sake of clearness, has not been drawn in. Blood flows from the artery (A) through the lymphoid tissue, and then through the pulp, in the direction of the arrows, *around* the lymphoid tissue, and then back through the lymphoid tissue again to the vein (V). The cells in that portion of the lymphoid tissue which immediately adjoins the pulp are beginning to differentiate. Er' = a lymphoid cell which is just beginning to acquire a coating of haemoglobin-containing cytoplasm and to develop into an erythrocyte. Er'' is a similar cell in a more advanced stage of development. Er = a mature erythrocyte in the pulp.

Fig. 5. Spleen of dogfish (*Scylliorhinus canicula*) stained with Leishman. E = an ellipsoid in transverse section. There are several other ellipsoids cut more obliquely. Note the blood in the lumina. LV = a blood vessel surrounded by lymphoid tissue. The lymphoid tissue is stained blue, the pulp red.

Fig. 6. Spleen of dogfish (× 250) washed out with normal saline and then perfused with Zenker-formol. S, S, S = pulp spaces in between the cells of the pulp reticulum and their processes. V = small vein formed by the coalescence of several pulp spaces. E = an ellipsoid.

Fig. 7. Spleen of dogfish (× 250) prepared by perfusion-fixation. *W* = wall of ellipsoid. *L* = lumen of ellipsoid. Two arrows have been drawn in the lumen of the ellipsoid, which is dividing into two and entering the pulp spaces. Compare with fig. 6. Figs. 6 and 7 together show that the circulation through the pulp of the dogfish's spleen is unquestionably an open one.

Fig. 8. Spleen of dogfish (× 250), showing junction between an arteriole (in a lymphoid sheath) and an ellipsoid. *WE* = wall of ellipsoid. *LE* = lumen of ellipsoid. *LL* = lumen of lymphoid vessel.

Fig. 9. Drawing of ellipsoid from dogfish's spleen; transverse section. *L* = lumen of ellipsoid. *P* = process from wall of ellipsoid to join with a cell of the pulp reticulum. *R* = reticulum cell of pulp. *Er* = erythrocyte lying in a space of the pulp reticulum. *Gr* = small pigment granules in wall of ellipsoid.

Fig. 10. Spleen of dogfish (× 500). High power view of lymphoid tissue. *H* = large hyaline cells, scattered among the smaller lymphoid cells. Note the cytoplasm of the hyaline cell, which is practically unstained.

Fig. 11. Cells from a smear preparation of dogfish's spleen (× 1500). Nos. 1–7 represent various stages in the development of the small round cell into the erythrocyte. Nos. 8–13 represent stages in the course of its development into the granulocyte. (Stained with Leishman's stain.)

Fig. 12. Section of dogfish's spleen, passing through the boundary zone. It will be seen that there is no sharp demarcation of the pulp, lying in the lower and right-hand portion of the drawing, from the lymphoid tissue lying above and to the left. The pulp is stained predominantly red, the lymphoid tissue blue. *Er* = two stages in the development of the small round cell into the erythrocyte. *Gr* = stages in the development of the small round cell into the granulocyte. The actual details of these changes are much better seen in smear preparations. The section is necessary however to show the micro-anatomical position of the cells.

Fig. 13. Spleen of dogfish. Frozen section stained with Sudan III (× 250). The yellow staining lipoid granules show up black in the photograph. *LG* = lipoid granules.

Fig. 14. Spleen of ray (× 250). In the middle of the field is an ellipsoid cut in transverse section. There is blood (showing black in the photograph) in the lumen. *Er* = an erythrocyte in the wall of the ellipsoid. Although it is not obvious in the photograph, in the actual slide the erythrocyte is quite obviously undergoing disintegration.

Fig. 15. Blood of *Gadus minutus* (× 625) showing triangular red blood corpuscles.

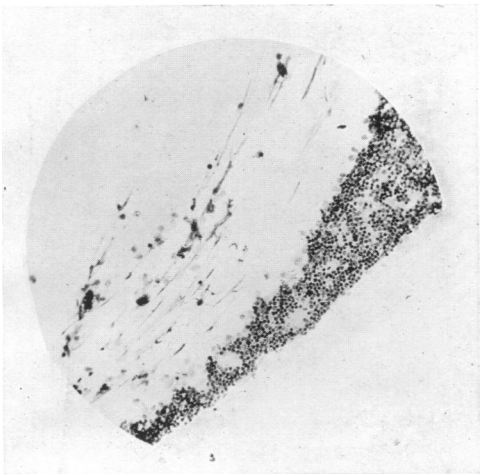


Fig. 1.

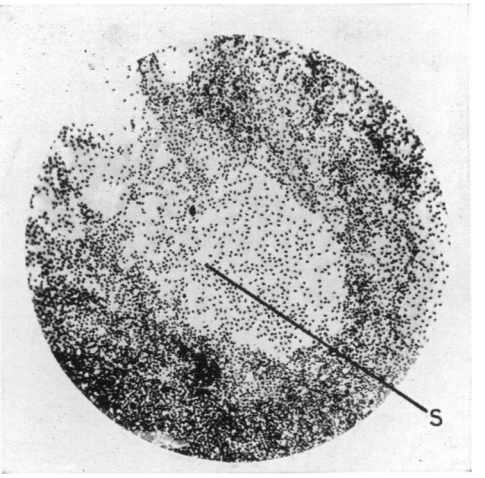


Fig. 2.

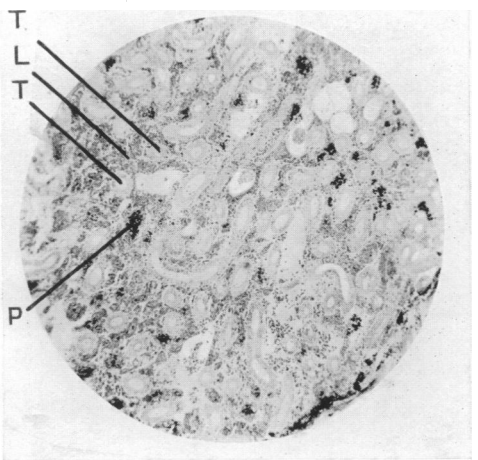


Fig. 3.

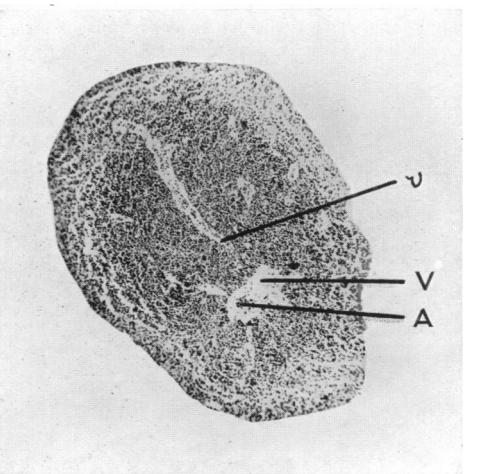


Fig. 4.

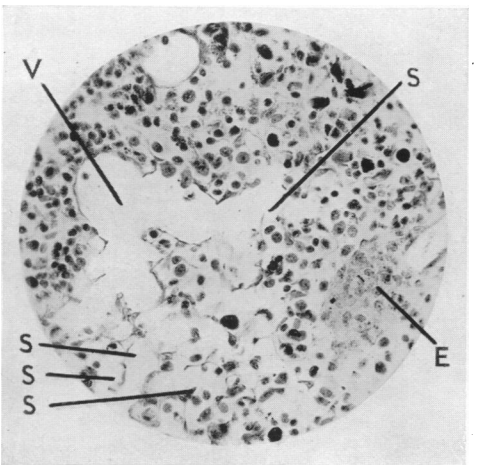


Fig. 6.

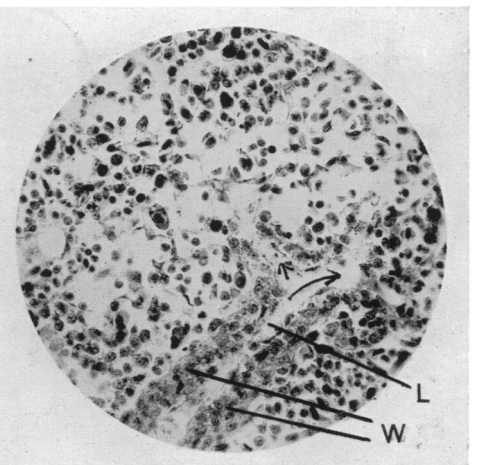


Fig. 7.

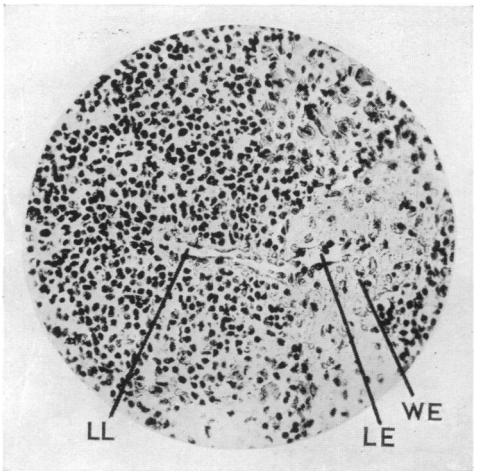


Fig. 8.

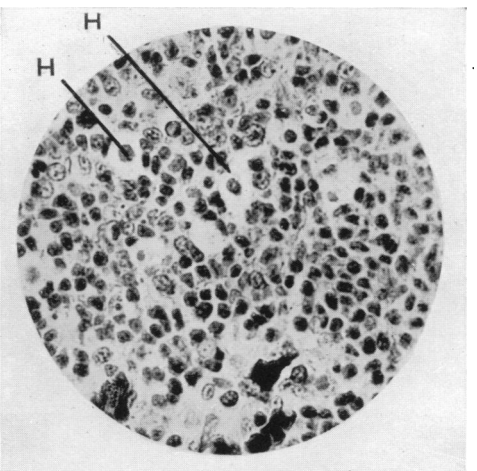


Fig. 10.

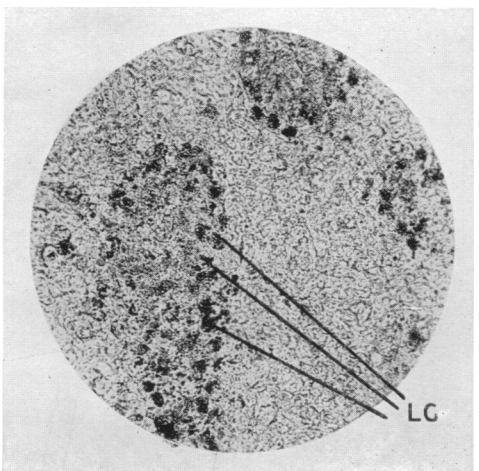


Fig. 13.

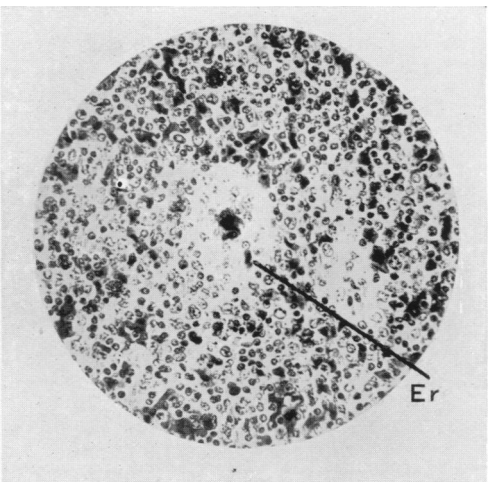


Fig. 14.

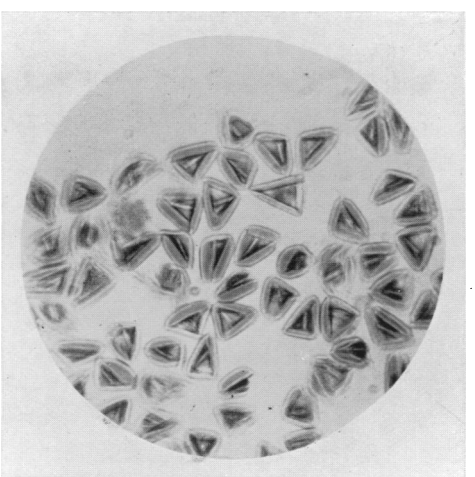


Fig. 15.

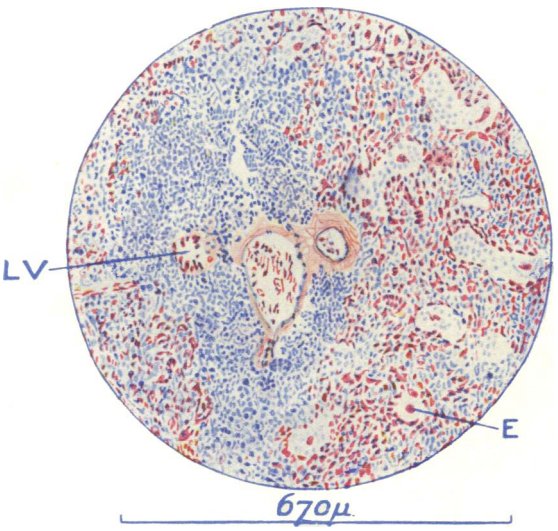
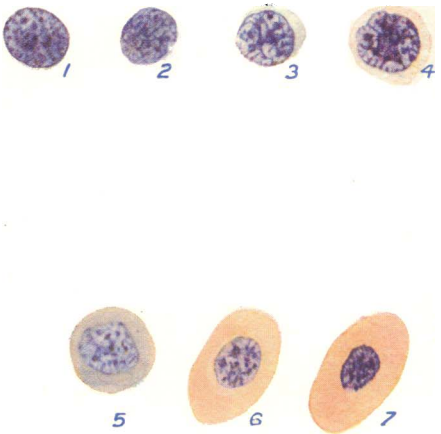


Fig. 5



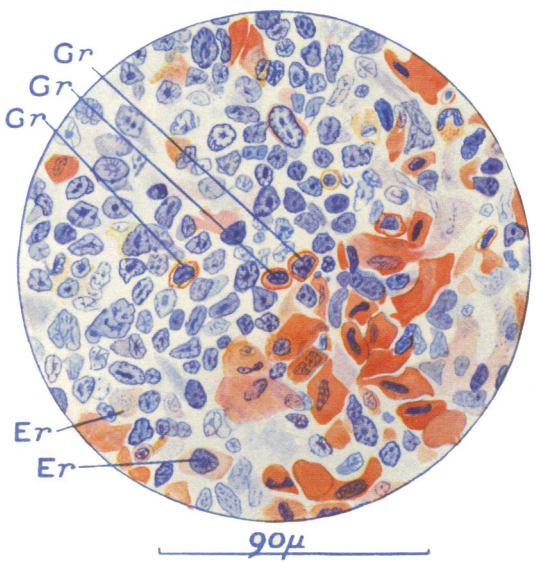


Fig. 12

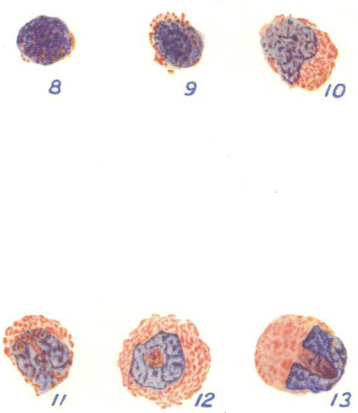


Fig. 11