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A Contribution to the Cytology of the Spleen: The Romanowsky-Stained Bovine Spleen Smear.

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Dedicated to JOSEPH GILLMAN, M.B., B.CH., D.SC., Professor of Physiology and formerly Senior Lecturer in Histology, University of the Witwatersrand, whose unremitting and distinguished researches in cytology have inspired and encouraged fellow South Africans in their investigations into the biological sciences.

PREFACE.

This work had its inception as long ago as 1934 when the senior author made sporadic observations on the cytology of spleen smears. From 1938 onwards it became a definite project, but interruption by other duties and intervention of war caused long delay in its completion. This caused difficulty in discussing the literature, some of which appeared after many of our observations had already been made. The immense volume of haematological literature forces us to give only a selected bibliography.

Both the present Director of Veterinary Services, Dr. G. de Kock, and his recent predecessor, Dr. P. J. du Toit, took personal interest in the progress of the work. To them our thanks are due for enabling us to undertake and pursue these studies.

To Dr. A. C. Allison, now of Merton College, Oxford University, special tribute is due for his arduous and brilliant labours on the illustrations which form an essential part of this monograph. These illustrations were ably supplemented by Miss G. E. Laurence.* Dr. E. J. Marais of the Council for Scientific and Industrial Research checked our test for planeness of surface of glass slides. We are greatly indebted to members of the staff of the Department of Microscopical Anatomy of the Medical School, Johannesburg, for fruitful discussions and useful opinions. Further acknowledgments are made in the text.

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* Who was responsible for figs. 9 and 10, 20 and 21, 38 to 40, 55, 106 to 108, 112, 113, 120 to 122 and 130 to 132.

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Chapter I.—Introduction.

It would be almost callous to omit from an article on spleen smears mention of what they have meant in South Africa in terms of human energy and patience. Since 1915, when the delivery of a spleen smear became officially regarded as adequate notification of death, as required by Government Notice 638 of that year, smear examination has constituted an increasingly important part of the routine duties of officers of the Division of Veterinary Services. In the years 1915-45 approximately nine million smears were examined,(1) of which the great majority would have been spleen smears. Mention of this aspect of the matter also affords the opportunity to explain that the first impetus to the present work arose essentially in an effort to relieve the tedium of routine smear examination by infusing some scientific interest into it, so that information of biological value—in addition to that required merely for the veterinary police control of scheduled diseases—might be accumulated in return for all the labour involved.

Later it became apparent to us that many of the problems of the routine smear could not be solved by passive observation of this type of preparation, but only by research methods, especially by improvements in technique. Thus the anomalous situation arose that our efforts to relieve the monotony of examining smears from the field resulted in our having to impose on ourselves the additional study of countless smears prepared by us in the laboratory.

In the case of the vast majority of routine spleen smears—submitted as a compulsory obligation from animals dead from any cause whatever, including clinically healthy subjects which have been slaughtered, no positive diagnosis can be made or is even anticipated. One searches through a jumbled mass of material derived from the spleen, interested only in the possible detection of parasites. But when intelligently appraised, this material is scientifically interesting and ultimately its study can become fascinating.

Moreover, the drawing of a smear actually accomplishes a microdissection of the elements, and in fortunate instances very beautiful and instructive examples of this process are to be seen. It is surprising, therefore, that after so many years of spleen smear examination no literature on the bovine spleen smear exists.(2) If one verbally compares notes with highly experienced smear examiners, one is little short of amazed at the varying interpretations placed on the very commonest appearances, when indeed they are analysed at all. Yet surely one can more credibly dismiss a given appearance as being of no pathognomic significance if one is able to say what it is, in addition to merely what it is not.(3)

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(1) In 1921 (*Jl. S.A.V.M.A.* I:39) the Director of Veterinary Services remarked: "We will simply have to change the present system. It cannot go on . . . The position has become intolerable to my mind . . . This smear work is paralysing all the activities at Onderstepoort". Nevertheless, 24 years were to elapse before (in 1945) it was first attempted to bring some relief to professional officers by training and employing laymen to assist in the work.

(2) In 1927 the late Dr. C. P. Neser, of this Institute, announced his intention of reading a paper on "Pitfalls in Smear Diagnosis," but at the meeting in question apparently thought better of it and obtained permission to change the title to "Smear Examination at Laboratories". In this and in the ensuing discussion, solely the administrative aspects of smear examination were dealt with. (See *Jl. S.A.V.M.A.* 1:36.)

(3) It would be an interesting psychological study to ascertain what chiefly occupies the minds of the long-suffering examining officers while at the microscope, but it would be safe to predict that this would not include the spleen as an organ!

Nevertheless, a great wealth of practical experience of the appearances of the splenic elements has doubtless accumulated, and experienced examiners often suspect a specific diagnosis long before they have demonstrated the causal parasitic organism, from an almost instantaneous and largely subconscious appreciation of cytological changes which they would certainly be at a loss to describe in technical terms. We believe the accurate analysis of such pathological changes in the spleen to be of considerable interest and importance, but unfortunately this study has had to be indefinitely suspended. In any case a preliminary necessity is to give an idea of the normal splenogram. However, we have not hesitated to mention pathological appearances where, by so doing, the normal cytology could be clarified or emphasised.

In addition to the value which the analysis of the spleen smear should have for the routine worker, there is its interest in connection with the normal cytology and especially the cellular physiology of the spleen. These aspects steadily increased in importance during the course of the work, until, as will be seen, they became predominant.

Evaluation of the Smear Method.

Particularly where blood-forming organs are being studied, the smear or imprint method offers well-known advantages over section methods. Striking illustration of this point has been offered by Kirschbaum and Downey (1937). But although many workers have emphasised the superiority of the smear method—e.g. more recently Erf and Herbut (1946)—it still does not enjoy the almost universal usage it deserves. It is almost a *conditio sine qua non* of Romanowsky technique, which is considerably and often incomparably more brilliant when applied to the air-dried smear than when used on sections. The smear method also largely avoids the shrinkage of cells so inevitable in sections, and which makes the subsequent recognition and accurate study of the elements a matter of great difficulty or even impossibility.([4])

Bloom (1928[a]) is very critical of the dry smear technique for organ preparations, inasmuch as staining is supposed to be very "faulty and atypical" with May-Grünwald-Giemsa and other methods. He cites Downey (1927) as also having encountered this difficulty. Yet this author clearly states (p. 158): "So far the work on marrow and lymphoid organs with the dry smear method has shown, first: the importance of this method for estimating the finer cytoplasmic and nuclear structures, particularly when it is desired to compare cells from the hemopoietic organs with cells from blood smears . . ."; and (p. 175): "The sections and moist smears do not bring out the finer details of structure, especially of the nuclei . . . It is true that cells prepared by this method do not look much like living cells, but their characters are so constant under like conditions that one must assume that their appearance is not wholly artificial".

Ehrich (1934) also stresses the value of dry smears. The only advantage of the wet-fixed smears is that nucleoli are better shown (Bloom 1928[a], Silberberg 1932). Hall (1938), in comparing the supravital preparation with the dry smear method as a routine laboratory procedure, is in favour of the latter. His critical evaluation of the supravital method is well worth while studying if one wishes to reconcile findings based on supravital technique with those derived from the study of smears. [Similarly, the recent study of Jones (1948) on the nuclear pattern of lymphoid cells in dry fixed smears merits attention.]

([4]) Bloom (1938a) has remarked on the apparent increase in size due to flattening of spherical elements (p.376), and has pointed out the importance of bearing this in mind when determining cell size in smears.

On the other hand, the routine method of preparing smears, consisting usually in scraping up some splenic pulp with the edge of one slide and smearing it over the surface of a second, is an execrable example of haematological technique, failing as it does to preserve the elements in a form approximating their living state. The cellular distortion and disintegration resulting from pressure, friction and tearing is extreme in this method of dislodging delicate cells from the imprisoning reticulum and spreading them over a slide: nuclei are torn from their cytoplasm and squashed into fibrillated masses or drawn into long strands, while torn off cytoplasmic fragments are left strewn about. Even with improved methods one cannot possibly hope to escape some degree of damage.

The problem immediately arises as to the correct assessment of (1) artefacts due to damage of normal structures, i.e. exogenously produced artefacts; (2) artefacts produced by mechanical damage to structures normally present but in incipient stages of senescence and/or degeneration, i.e. endogenously conditioned artefacts; and (3) appearances resulting from certain physiological processes, but simulating artefacts whether exogenously produced or endogenously conditioned.

Some selections from the literature may be given to illustrate the nebulous state of our knowledge regarding this aspect, based as it is on a few casual observations and personal opinions.

Tempka and Kubiczek (1938) describe and illustrate formation of "naked lymphocyte nuclei", squashed nuclei and chromatin smudges and networks as due to a pre-existing state of degeneration, arguing: ". . . . wenn also ein Teil der zellulären Gebilde die Form der nackten Kerne annimmt, so ist darin eben der Beweis zu sehen dass die betreffenden Zellen besonders empfindlich, weniger resistent sind, eine 'fragilité particulière'". The expression is a quotation from Weil, Isch-Wall and Perlès (1936) (p. 22): "Les noyaux nus existent toujours en grande quantité dans toute ponction de rate; le traumatisme que l'on fait subir à la pulpe splénique explique leur présence. Ils sont dus probablement à une artifice d'étalement et à une fragilité particulière des éléments; on peut les mentionner dans le splénogramme, mais ils doivent en aucun cas faire partie du pourcentage." Fontana (1925) (cit. Spadolini) also observed this so-called selective unmasking of cells in incipient stages of degeneration by the making of dry preparations. Osgood (Osgood and Ashworth—1937) considers squashed cells as "remnants of dead cells".

Sabin (1923) and Sabin, Cunningham, Doan and Kindwall (1925) regard fragmented neutrophils with scattered granules as being the result purely of mechanical damage. Naked nuclei with pale-staining shadows of granulations in dry smears are, however, thought to correspond to non-motile (i.e. degenerating) leucocytes of supravital preparations. Yet this criterion was not found to be infallible. In fact, Sabin and Doan (1926) state (p. 833): ". . . more leucocytes are fragile in the technique of pulling films than are actually in the non-motile phase of the supravital technique". Apparently Whitby and Britton (1935) loosely extrapolate this standard to apply to other appearances and other cells as well; for example: "Basket cells" (from granulocytes), and "Smear cells" (smudges derived from lymphocyte nuclei). (Compare their plate 3, page 36, figs. O and P with our plate VI, figs. 111 and 107).

Apart from these non-motile forms of neutrophils, phases of degeneration have been described for lymphocytes, i.e. decreased number of mitochondria (Sabin, Cunningham, Doan and Kindwall, 1925); active extrusion of lymphocyte nuclei

from the cytoplasm (Kubie and Schultz, 1925), whilst Sabin (1923) casually mentions forms thought to represent damaged or degenerating monocytes, as well as naked lymphocyte nuclei. One cannot deny their existence, nor belittle the susceptibility of these cells to physical trauma; yet in the absence of careful quantitative determinations and more detailed qualitative observations, one is at a loss to assess their importance in producing artefacts in the dry smear.

As a consequence, interpretations may be rather haphazardly applied. For instance, Spadolini (1928, 1929) regards certain nuclear smudges, or the formation of fine chromatic filaments unassociated with granular detritus, as being due to the preparation of the smear. But various states of squashed, fragmented or frayed-out nuclei (" ombre o placche reticolato ") are regarded as pre-existent in the normal spleen. Indeed, the juxtaposition of granular material to, or superimposition on, such nuclei is taken as proof of the origin of platelets from such " disintegrating " nuclei of " splenocytes ". This author observed the presence of platelet material in cytoplasm and even in the nuclei of cells, and correctly interpreted this as phagocytosis. But as soon as the cell assumes a damaged appearance in smears, this is again accepted as proof of the nuclear origin of platelets!

In our experience lymphocytes are undoubtedly the worst sufferers, even considering their overwhelming superiority in numbers in the spleen smear.[5] Denudation of the nucleus is the most common occurrence met with; smudging, and drawing out of the nucleus into filaments, being respectively next in frequency. The large lymphocytes are, remarkably enough, more immune; they seem to obey an " all-or-none " law; they are either damaged almost beyond recognition or show at the most a few wisps of cytoplasm torn off. The fragility of the lymphocyte is explained by the findings of MacKenzie, Whipple, and Wintersteiner (1941), who observed this type of cell to have but a limited compressibility as compared with the other cell types seen in living transilluminated mammalian spleens. It is quite conceivable that cells exhibiting differences of physical and physico-chemical characteristics will react differently to what one must appreciate is a major traumatic influence, even under the most favourable of circumstances. Thus we find that plasma cells, in contradistinction to the opinion of Weil, Isch-Wall and Perlès, are usually seen intact, even though the nuclei show unmistakable evidence of degeneration (figs. 47, 49 and 50). In our opinion, " fragilité particulière " is applicable more to different cell types than to different phases of the same type of cell.

When a micro-population of cells is subjected to a process of flattening and smearing a host of minor factors will come into play, and will determine the total traumatising force exerted on each cell. Considerable variation from cell to cell is to be expected. The variations between smears made by different techniques, and between films successively prepared from the same material by the same technique, amply illustrate this.

Apart from the results of actual damage, certain physiological processes may give rise to products which resemble artefacts. A proportion of the numerous cytoplasmic fragments and masses noted in smears may represent actively shed " surface films " from macrophages (Sabin 1939, and Schain 1945); or they may represent fragments budded off from neutrophils *in vivo* (Sabin 1923), or from

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[5] In peripheral blood smears from humans and rabbits Sabin, Cunningham, Doan and Kindwall (1925) found on the other hand the neutrophil to be the most fragile cell: damaged lymphocytes they found in less than one per cent. of cases.

lymphocytes (Downey and Weidenreich 1912, White and Dougherty 1944, Dougherty and White 1945); or even the platelet-like fragments from reticulum cells, sheath cells and endothelial cells as described by Watzka (1937). We have seen numerous cytoplasmic fragments and globules in spleen sections, most distinctly after moderate perfusion. What we interpret as active extrusion of cytoplasmic globules (cf. Ranvier's "clasmatocytes"; Renaut's "cellules rhagiocrines") rather than shedding of surface films was observed in mobilised reticulum cells in sections from the spleen of a cow suffering simultaneously from *Babesia bovis, B. bigemina, Anaplasma marginale* and *Theileria mutans* infections. In supravital preparations too, formless masses of debris have been seen (e.g. Sabin, Cunningham, Doan and Kindwall, 1925).

To sum up, we are of opinion that no matter how impossible the distinction between the various types of "artefacts", the majority are exogenously produced. The difficult question of "artefacts" in spleen smears is only to be appreciated by a study of good smears.

The other limitations of the smear method are obvious. It offers no opportunity of studying cells in their normal contiguity or juxtaposition. The relative numbers of various elements seen are greatly influenced by the care with which they are dislodged. The quantitative evaluation of the normal splenogram is consequently subject to greater error than for instance an ordinary or differential blood count.

The smear method thus constitutes one avenue of approach in morphological study, and a very useful one it is indeed; but ultimately the results obtained have to be integrated with those obtained by other methods.

Problems of Terminology.

Very considerable problems of terminology have thrust themselves upon us throughout the present work, some of them of fundamental biological importance. There appears to exist no uniformity of principle in the naming of cells. Nor are guiding rules laid down, as for example in the naming of species. To us it seems that such principles are badly needed in histology. Often a cell has been named in ignorance of its origin and life-history. When such information about the cell later becomes available, may the name be changed, and if so, under what circumstances? The original name for example may have been uninformative or actually misinformative; and while being conservative about revising nomenclature one cannot but hesitate to acquiesce in the perpetuation of errors.

Indeed one is constantly on the horns of a dilemma: viz., reluctance to multiply names (which in any case may not meet with acceptance) versus unwillingness to continue with the use of names which, although generally accepted, have become intimately bound up with theoretical considerations and which either implicitly or by inference carry erroneous or misleading implications about cell derivations.

In the last analysis one has to remember that terminology should be the servant and not the master of cytological knowledge. However, in haematology one has to admit that the latter state of affairs has sometimes tended to prevail; and one is entitled to ask whether the time has not arrived for a fundamental revision, or at least that agreement should be reached on the principles of nomenclature of metazoan cells, whereby future revision could be undertaken piecemeal but on a uniform set of criteria.

Yet the whole situation bristles with difficulties. Many would probably admit that the practice of assigning distinct names to what are merely stages in the life-history or successive physiological phases of one and the same cell is undesirable, e.g. lymphocyte→plasma cell. And one may fairly readily anticipate that a well-documented plea for simple reform in such cases would find considerable support (e.g. the use of some such term as hyperplasmatic or ultrabasophilic lymphocyte, or better still, to use a histochemical characterization). But if such a system of reform were to be thoroughgoing, one of the most obvious cases where difficulty would arise would be in the nomenclature of the red cell series. Here is an example of a life-history perhaps the best attested of any in haematology; yet each stage receives a different name. One need not labour the apprehension that fundamental reform would stand no chance of acceptance here, in the face of deeply established usage, which is moreover intimately bound up with everyday clinical considerations.

Another difficulty has already been touched on by implication, viz., that reform, if undertaken, must inevitably occur in piece-meal fashion; since nomenclature, if it is indeed to be the tool of cytological knowledge, must be assigned by those whose acquaintance with the particular cell types is the most accurate and specialised, and must come about in the case of each cell type as and when such precise information becomes available. Hence a conference of scientists could hope to achieve little, and a standing committee of workable dimensions would be necessary. But could such committee hope to be truly representative? It certainly seems that some machinery for guiding or controlling reform will sooner or later have to be established. And the later it is set up the more the confusion and wasted individual effort that will meanwhile have accumulated.(6)

On the other hand, it is quite conceivable that some authorities would hold the view that there is nothing undesirable in having different names to describe temporary appearances of a single cell, this indeed being the position that commonly obtains and which hitherto one has had tacitly to accept. Should this opinion prevail, one would have the objection that—leaving aside its wisdom—there is nevertheless no uniformity in this practice. One might ask: "Why then do not for example an azur-granulated lymphocyte or a Russell-body-filled plasma cell receive distinctive names?" If the reply to this is: "Because they remain recognisable for what they are," it draws the rejoinder: "But surely an erythrocyte and a neutrophil are recognisable, viz., respectively as a normoblast that has lost its nucleus, and a matured myelocyte. Why are these singled out to enjoy special names?"

It will thus be seen that in cytological nomenclature two mutually inconsistent principles are applied; and further that the choice of application of these is haphazard and dependent on customs established at a time when our knowledge was far more fragmentary than it is to-day. There is a consensus of opinion neither on (1) *what the function of a name should be* (i.e.) in how far should it serve to summarise our knowledge of the cell which it connotes) nor even (2) *whether indeed a need exists for uniformity* of terminological practice.

We have been very conscious of all these difficulties in deciding under what names to describe the cells of the spleen. They arose in most acute form when considering the most common phagocytosing elements of this organ in the ox. For these the term "macrophage" is probably the most firmly established. But

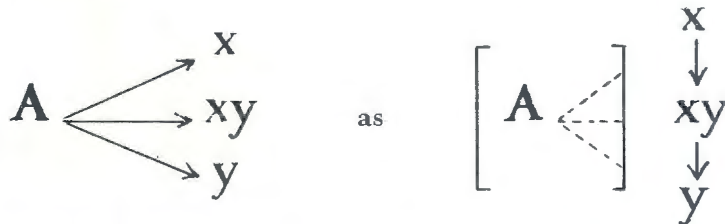
(6) Since this was written the "First Report of the Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-forming Organs" has appeared. (*Am. J. Clin. Path.* 1948 *Vol.* 18. *No.* 5, *pp.* 443-450).

it is uninformative and undoubtedly has come to denote attributes in strong contrast with those commonly ascribed to lymphocytes. When we found that this cell was nothing but a lymphocyte in a phase of phagocytic activity, we could not bring ourselves to continue with the use of so misleading a term. We were subsequently relieved to find that we were in good company (Taliaferro and Klüver, 1940), in boldly referring to these cells as phagocytosing lymphocytes. Nevertheless, we probably remain inconsistent in other directions, e.g. in using the term plasma cell for certain elements which we have no doubt to be phases in the existence of reticulum cells. One does not know where to stop: lymphocytes may also be regarded as phases of reticulum cells. For that matter, extreme unitarians regard the erythrocyte as a phase of the lymphocyte. One dare not embark on reform in such cases.

Problems of Cell Derivation.

The criterion of the existence of cells transitional between one type and another has been extensively used, more especially in haematological cytology, as evidence or even as proof of a derivation of the one from the other. But it seems to require strong emphasis that the mere occurrence of cells "xy" intermediate in morphological characters between one cell "x" and another "y", is inadequate evidence of a derivation of "y" from "x", *unless it can be excluded that both "x" and "y" may be derived from a common source "A"*.

Thus it is only too easy to miscontrue the relationship:



The occurrence of cells intermediate in character between lymphocytes and monocytes has been widely used as evidence of the lymphocytic derivation of the monocytes. But if, as we believe, both are derived from the reticulum cell, it is quite to be expected that the latter might sometimes give rise to a cell of intermediate or uncertain character. Bovine blood is notorious for the difficulty it has caused observers in discriminating between lymphocytes and monocytes (*vide* Kohanawa, 1928). We have always felt that this difficulty was exaggerated and depended largely on paying insufficient attention to nuclear texture. But the occurrence of genuinely unclassifiable cells need not disconcert us.

Problems of cell derivation arise in acute form when studying any blood-forming organ; similarly when studying pathological blood in which elements ordinarily confined to the haemopoietic organs have untimeously found their way into the bloodstream. These problems link up with and vastly complicate the obstacles to a rational or even intelligible nomenclature discussed above. Indeed, the very act of assigning names to cells savours of an insufficient appreciation of the dynamic state of affairs existing among the cells of the blood-forming organs: it is liable to engender a static conception of the cellular elements and tacitly

to encourage ideas of an immutability of types, such as is implied in the naming of species. Yet names, as Oettlé (1947) reminds us, are unavoidable, and static conceptions must be fought largely by other means.

Material and Technique.

Of the routine smears mentioned previously, some 50,000 were examined by the authors during the period this investigation was in progress. They originated chiefly from the so-called " East Coast fever areas ", i.e. from certain low-veld areas of the Province of Transvaal, and to a lesser extent from similar areas in Natal. From this mass of moderately well to poorly prepared material, a rigorous selection yielded about a hundred more fortunate preparations suitable for intensive study or for the demonstration of certain artefacts.

As previously indicated, this material had to be supplemented by more carefully made smears. Merely drawing a slide over the cut surface of the organ was not found to be much better than the routine method. The impression method (touching) yielded preparations, which, although quite good or even excellent in parts, were still far from satisfactory considering the preparation as a whole.

Buffering the mechanical forces of distortion, and thus approaching the conditions obtaining during the making of a blood film, naturally suggested itself. For this purpose serum or oxalated plasma was found the best. Endicott (1945) recommended the use of plasma or serum for making bone-marrow smears. Although from long experience we can fully endorse this recommendation, it seems seldom to be bothered about in pathological laboratories. Ringer or Ringer-Locke solution serves this purpose very poorly. No critical experiments were carried out to determine whether heterologous, homologous, or even autogenous serum or plasma is to be preferred. From general considerations, autogenous plasma was used whenever possible, being obtained from blood collected just before slaughter or during bleeding-out of the carcass, and centrifuged while the spleen material was being taken. When this was not possible, recourse was had to a supply of homologous serum or plasma which had been collected under sterile conditions, passed through a Seitz filter to remove any cellular elements or chance contaminants, and stored at 0° C. This was filtered through fine filter paper immediately before use to remove any sediment or cloudiness(7). In the case of plasma, sufficient potassium oxalate was used in the collecting vessel to prevent coagulation of about $1\frac{1}{4}$ times the volume of blood collected, thus providing a margin of safety against the eventuality of coagulation during the subsequent manipulations. To prevent undue dilution of plasma a fairly concentrated solution of oxalate (10 or 20 per cent.) was preferred.

With regard to the spreading of the film and the ultimate distribution of cells on the slide, there exists an optimum although comparatively wide range of relative density of the splenic elements in the final suspension. Since speed is an important factor in the preparation of these films, and the relative density may be greatly influenced by the blood content of the spleen from which the sample originated, no attempt was made at careful determination of the volume of spleen pulp and that of the serum or plasma used as suspending medium. Reliance was rather placed on practical judgment which was soon attained. The final suspension should appear to the naked eye like hydraemic blood in colour and like leukaemic blood in consistence.

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(7) Occasionally, when serum was used, small coagula would hamper the smear-making at a critical stage of the proceeding. This may be due to admixture with the plasma of the selected sample of the splenic pulp.

In order to remove any particulate matter which, on account of its size, would prevent the smooth spreading on the slide, a filter of sintered glass was eventually found indispensable. As such filters were unobtainable during wartime, Messrs. S. D. Rossouw, B.Sc. and J. H. Minné, B.Sc., of this Institute kindly undertook to manufacture some. By trial and error the most suitable filter disc was found to be of very coarse type (roughly between 1G and 2G in degree of coarseness), measuring 1·5 to 1·75 cm. in diameter and 2·0 to 2·5 mm. in thickness, made by fusion of hard glass particles between 0·4 and 0·5 mm. in average diameter. The disc was fused into a Pyrex or Monax test tube, one end of which was then cut off level with the disc and the other about 3 cm. from it.

Regarding the actual spreading of the film, a method similar to that used in making blood films for differential counts is best. But instead of using an ordinary slide or heavy groundglass slip as popularised at this Institute by Neser (1923), we preferred a 30×40 mm. coverslip. This is gently lowered to a drop of spleen suspension placed on a slide, thus allowing the suspension to spread by capillary attraction only. Before the coverslip comes into contact with the slide (i.e. before atmospheric pressure comes into play) it is gently swept off, stricly parallel to the surface and without exerting any pressure whatsoever. As an additional refinement, plane-surfaced slides and coverslips were used.(8) The slides were chemically cleaned, stored in acetone-alcohol, and polished with silk immediately before use.

Finally, rapid drying is essential. This was secured by the use of an electric hair-drier.

The ultimate result was much better than that obtained by the usual means, and in favourable instances the preparations were comparable with the best of blood films. It must be realised that on account of differences in size and numbers, one cannot simultaneously obtain optimal spreading of both red cells and nucleated cells. When the former are perfectly spread the latter are damaged unduly; when the latter are well prepared, the erythrocytes are crenated or incompletely spread.

Efforts to simulate the cell's normal physical environment during the manipulations, by preparing the suspension in an atmosphere saturated with water vapour and kept at 37° C., yielded results which by no means justified the additional labour involved.

In practice the procedure was as follows: The spleen was removed or a piece cut off as soon after death as possible. The capsule was removed from an area of about 3×2 cm., a block of splenic tissue approximately 0·5 c.c. was cut out from the depths of the organ and immediately transferred to a watch-glass containing approximately 0·5 ml. of bovine serum or plasma, where it was rapidly pulped by cross-cutting movements with two lancet-tipped dissecting needles. The mash was then poured through a sintered glass filter and a drop of the emanating translucent suspension picked up with a clean platinum loop and placed on a clean, plane-surfaced slide. A coverslip was gently lowered on

(8) A simple method of selecting plane-surfaced slides from amongst a batch of ordinary ones is to move each back and forth, and up and down, about two feet from a page of print, or before the observer's eyes while viewing a distant unplastered brick wall or corrugated iron roof. Any distortion indicates a reject. Strictly speaking this is a test for parallelism, but for all practical purposes there is a reasonable degree of correlation with the interferometric method. A simple apparatus for slide-testing has now been devised. (Marais—in preparation).

the drop to avoid bubbles, and after allowing a second or so for the suspension to spread, was slid off as explained. Rapid drying in front of an electric drier completed the procedure.

Staining was done by the May-Grünwald-Giemsa (Pappenheim) method. Modifications introduced during the course of the work resulted in the following procedure:—

(1) Fixation in methyl alcohol, 6 minutes.

(2) May-Grünwald stain plus distilled water, 6 minutes.

(3) Without washing transfer to Giemsa stain 7 ml. per 100 ml. of fresh, triply distilled water, plus 7·5 ml. acetone as recommended by Lillie (1944). Stain for 25 to 35 minutes at 56° C. (i.e. in the embedding oven).

(4) Rinse in triply distilled water buffered to pH 6·2 for 30 seconds.[9]

(5) Wash rapidly in ordinary distilled water and dry.

Coplin jars in which the slides were kept upright were used to prevent stain precipitate.

Smears made in the ordinary way were kept for comparison, including preparations made from the serosa and other parts of the peritoneum to study the cytology of the serosal cells.

Material fixed in 10 per cent. formalin and in Zenker-formol was embedded in paraffin or celloidin-paraffin, and stained with haemalum-eosin, van Gieson, or Giemsa, or impregnated by Gordon and Sweets' modification of the Bielchowsky technique for reticulin, as required. More valuable information was gained by perfusing the spleen with saline, followed by Zenker-formol perfusion. Starting the perfusion within fifteen minutes after death and using fluid at body temperature greatly facilitated proper washing out of free cellular elements. On account of its size, complete perfusion of the bovine spleen was not obtained. By dissecting along the splenic artery the best parts were selected for embedding and sectioning. About 4 L. of perfusing fluid followed by 0·5 L. of fixative was used at a pressure of 130 cm. of water.

Excellent sections were obtained from Helly-fixed material, double-embedded in celloidin-paraffin and stained with Giemsa ($2\frac{1}{2}$ drops per ml. of distilled water with addition of acetone as before) for 6 to 8 hours at 56° C. The stain should preferably be changed once. The sections were differentiated in 96 per cent. alcohol to which a few drops of a saturated acetone-alcohol solution of colophonium [10] had been added to give a light straw-coloured solution, and dehydrated in three changes of absolute alcohol, followed by clearing in xylol and mounting in cedarwood oil.

Connective tissue fibres were recognised by applying selective methods (silver impregnation for reticular fibres, orcein for elastic fibres, etc.), suitably modified for use on smears. The Prussian blue reaction was made use of in the recognition of haemosiderin and "haemofuscin" (see under the heading "Sinus-lining Cells" for further details regarding the latter pigment), and Sudan IV and Sudan Black for possible presence of fat.

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[9] Concentration of Giemsa, time of staining, pH of the subsequent wash in distilled water, all had to be eventually adapted anew to every batch of stain. Sometimes step (4) was found unnecessary.

[10] We find that differentiation in acetic-alcohol, as is commonly practised, ruins Romanowsky-stained sections; indeed this fully accounts for their previous unpopularity at this Institute. The good Romanowsky section seems all too seldom to be seen in histological and pathological laboratories.

Isolation of Schweigger-Seidel sheaths from pigs' spleens, as mentioned on page 97 was accomplished by subjecting 1 mm. strips of fresh spleen to a fine strong stream of pig's serum from a syringe fitted with a fine hypodermic needle. From such a washed sample either a group of penicilli was removed under the dissecting microscope and stained and examined as squash preparation, or one or two penicilli were placed on a slide and gently smeared.

Chapter II.—Description of the Splenic Elements in Romanowsky-Stained Smears.

The Intact Elements.

(1) *Lymphocytes* (Plate I, figs. 1-10).

Lymphocytes (inclusive of phagocytosing lymphocytes to be discussed immediately below) are usually the predominant cells of the spleen smear, although sometimes they may be outnumbered by plasma cells. As mentioned elsewhere, the ordinary smear technique produces extensive artefacts in them: firstly, in nearly every cell the cytoplasm is more or less completely torn away from the nucleus, producing the appearance of "naked nuclei"; secondly, many of these nuclei are further damaged through crushing and tearing.

The intact lymphocytes as seen in efficiently prepared films need not be described in full detail. Medium-sized lymphocytes (fig. 2) with inconspicuous nucleoli predominate.[11] Small lymphocytes (fig. 1) with more trachychromatic nuclei and without visible nucleoli[12] are second in number. Large lymphocytes (lymphoblasts of dualists, or prolymphocytes) (fig. 3 and 4) are relatively few in number in the normal spleen smear.[13] Their nuclei are larger and more leptochromatic and amblychromatic than those of the medium lymphocytes, and contain prominent bluish staining nucleoli. In all these cells the cytoplasm is transparent and moderately basophilic, varying from sky blue to greyish blue. It may contain finer or larger chromophobe vacuoles (see fig. 94 where they are illustrated in free lymphocyte cytoplasm) and the so-called azur granules, either fine (fig. 5) or coarse (fig. 6), but which in the bovine tend to be coarser and more prominent than in most other species. Binucleate lymphocytes (fig. 7) and cells with lobed nuclei (fig. 8) (apparently due to amitosis—commonly termed Rieder cells) are seen. Mitotic figures are not as a rule encountered in spleen smears from clinically healthy animals. In diseased states (e.g. East Coast fever) or during periods of active immunisation to various protozoal infections, they are fairly frequent, and may even be found in the peripheral blood (fig. 9).

Lymphocytes may reveal a metamorphosis resulting in increase of amount and loss of basophilia of the cytoplasm. So pale does the cytoplasm become that neighbouring structures or artificial folding of the cell profile at first sight seem to form the delimitation of the cell outline (fig. 10). The nucleus becomes irregularly quadrilateral in outline and finally assumes the same bizarre shape as that of the phagocytosing lymphocyte described hereafter. Such cells are more resistant to mechanical damage. Their grouping in fairly close proximity to one another at

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(11) As Bloom (1938a) has pointed out, these cells are identical with the so-called "large" ("leucocytoid") lymphocytes of the blood. It should be noted that large lymphocytes (lymphoblasts) do not occur in the blood under conditions of health, but only small and medium lymphocytes, the latter especially in ruminants.

(12) It may be mentioned that small lymphocytes do possess nucleoli, as may be demonstrated by appropriate technique.

(13) In the hyperplasia of East Coast fever these cells together with intermediate stages of their derivation from reticulum cells, are increased and indeed usually overrun the picture.

definite sites in smears prepared in the ordinary way, leads one to suspect their possible association with the secondary nodules. Whether they represent lymphocytes that have phagocytosed microscopically subparticulate matter, that have completed the process of intracellular digestion, or have imbibed fluid, or, if the assumption of their intrafollicular position is correct, whether they represent spent elements (Sjövall, 1936), are speculations requiring further research.

2. *Phagocytosing Lymphocytes* (Plate I, figs. 11 to 21).

Much to our surprise when first we reached the conclusion, *the predominant phagocytosing element of the bovine spleen proves to be nothing but a lymphocyte.* At that time we had not read the work of Taliaferro and Cannon (1936), Taliaferro and Mulligan (1937), and Taliaferro and Klüver (1940), who arrived at the same conclusion from their study of human and simian malaria. Lymphocytes, perhaps especially small lymphocytes, are the cells chiefly engaged in the phagocytosis of erythrocytes. Furthermore, these same cells are highly active in the phagocytosis of blood platelets. *This phenomenon and its importance are only to be appreciated in smears prepared by meticulous technique.* In ordinary smears the cytoplasm of the phagocytosing lymphocytes is almost invariably torn away from the nuclei. We have no doubt that purely technical difficulties have been responsible for the current ignorance about the fate of the blood platelets and also for the confusion regarding the nature (" macrophage ", monocyte, " splenocyte ") of the most active phagocyte of the spleen. For discussion of this point refer to chapter IV, subheading 1, page 112.

Phagocytosing lymphocytes differ in no way—in either their nuclear texture or cytoplasmic appearance—from the ordinary lymphocytes. They may be small, medium or large. They phagocytose erythrocytes (fig. 11) or blood platelets (figs. 12, 13, 14, 15) or both (figs. 16, 17, 18). These phagocytosed inclusions indent the nucleus and cause it to assume an eccentric position. With increasing phagocytosis (fig. 18), the cytoplasm is stretched larger and larger in order to accommodate its contents. Both erythrocytes and platelets may, as stated, be phagocytosed by one and the same cell; more usually a lymphocyte contains either the one or the other. Phagocytosed platelets may not only deeply indent the nucleus, but may even appear to cause an actual intranuclear vacuole (figs. 14, 21). Evidence of phagocytosis of leucocytes by splenic lymphocytes may be seen (fig. 19). Haemosiderin may also be found in the phagocytosing lymphocytes, either alone or in conjunction with other inclusions (figs. 20 and 21).

The significance of these observations on phagocytosing lymphocytes will be discussed later in connection with the monocyte-macrophage problem (chapter IV).

3. *Plasma Cells* (Plate II, figs. 22-51).

Morphologically a plasma cell is characterised chiefly by its eccentric nucleus, its deeply basophilic cytoplasm and the presence of a prominent juxtanuclear light-staining area, the cytocentrum. Although our observations have convinced us that there is more than one sort of cell (viz. lymphocyte) which may undergo a change leading to acquisition of these characteristics, it seems advisable for practical considerations to deal with all such cells under this heading, and to leave open the question of the desirability of distinguishing nomenclaturally between " plasma cells " of varying origins.

Under normal conditions the majority of plasma cells in the spleen appear to be derived as a direct transformation of lymphocytes. There exists a perfectly graded series between the two types. These plasma cells correspond in size

and nuclear appearance with the three kinds of lymphocytes, so that one can readily distinguish small, medium and large (" lymphoblastic ") plasma cells (so-called " plasmablasts ") (figs. 22, 23, 24, 25 and 26). They are present in variable numbers, and are often exceedingly numerous in clinically healthy animals.

However, more especially in pathological states, (e.g. Quarter-Evil— *Cl. chauvoei* infection—and certain piroplasmoses) a large or even preponderant proportion of the plasma cells are obviously derived from the reticulum cells (fig. 27), or from cells transitional between reticulum cells and monocytes (" monoblasts ") (fig. 28, which illustrates an early stage of this transformation).

Such cells at first possess the nuclear characteristics of the types mentioned—amblychromatic, leptochromatic nuclei with " smoky " reddish brown karyosomes and often pale bluish multiple plasmasomes—but as they undergo ageing the increasing nuclear pyknosis obscures the features dependent on their different origin, and finally makes it impossible to assign a mode of origin to any given aged plasma cell, except by surmise from the preponderant evidence of derivation found in the smear in general. The centrosome may often be distinct in cells of this type (fig. 28), although not restricted thereto (figs. 25, 26 and 36).

Since making these observations on the origin of the plasma cells we find that it has been suggested in part by Parsons (1943) (from proliferating reticulum cells); by Lowenhaupt (1945) (from monocytic or histiocytic cells) and by Fagraeus (1948) (from R. E. cells). Naegeli (1923) mentions the appearance of so-called " myeloblastic plasma cells " as very rarely occurring in the blood; in acute myeloses they are more common, but their numbers remain inappreciable. Already in 1912 Hertz had pointed out the connection between the lymphoblastic type of plasma cell (Schridde type), and the so-called splenocytoid plasma cell (Hodara type). In 1929 Epstein had described the development of the plasma-like cells (" basophilic round cell ") from histiocytes (Kupffer cells and perivascular histiocytes of periportal tracts) in rabbits of which the R.E. system had been stimulated by non-infective antigens. Tempka and Kubiczek (1938) adopt this view, citing Naegeli, Rohr, and Sabrazes in support.

Definite morphological descriptions of this origin of plasma cells have lately been given by Bessis and Scebat (1946), by Jackson (1949) (in avian gliomata—the process being well illustrated), and by Kolouch, Good and Campbell (1947) (in bone marrow). This line of development was clearly brought out under conditions of abnormality or stress (hyperplasia and malignancy, sensitisation and subsequent shock, respectively) in their cases, as it was in our material. Bessis and Scebat actually distinguish between a slow rate of differentiation, which develops via a monocyte stage, and increasingly rapid rates in successively more severe degrees of hyperplasia and malignancy, whereby the intermediate stages are telescoped to omit a definite monocyte phase. All the authors just mentioned, with the exception of course of Naegeli and Jackson, either directly or by implication deny the lymphocytic origin of plasma cells. Only Rohr (1936) leaves open the relation of the " reticulum plasma cell " to the " blood plasma cell."[14] Forkner (1930) is against the monocytic origin of plasma cells, on account of the absence of the " segregation apparatus ".

The cytoplasm of the splenic plasma cells often contains numerous unstained vacuoles (fig. 29). Between these and the less frequent Russell bodies there exist all gradations, (figs. 30, 31) leaving no doubt as to the origin of the latter as a cytoplasmic product of the plasma cell.

An old theory, which Brown (1910) attempted to revive was that Russell bodies are phagocytosed erythrocytes. In addition to the different staining reaction with Romanowsky stains mentioned below, McConnell and Lang (1920-1) have pointed out that, unlike erythrocytes, they are Gram-positive. This also refutes Jordan's (1929) idea of Russell bodies as representing abortive attempts at haemoglobin elaboration by plasma cells. An obvious suggestion is that Russell bodies represent the gel-phase of the fluid vacuoles of plasma

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[14] The old question of the fibroblast origin of the plasma cells cannot be re-opened here, but see Miller (1931) and Kingsley (1924) as well as Michels' (1931) review.

cells and thus may represent a storage phase in plasma cell secretion. This possibility was considered by Downey (1911) and by Apitz (1940). Brass (1943a) considers only the vacuoles to be the morphological evidence for (protein) secretion, but the Russell bodies and their "initial stages" as signs of resorption of proteins foreign to the blood (or tissue). This latter view is also shared by Alexeieff (1925a) who regards Russell bodies as indicative of selective absorption of toxic products.

The Russell bodies (figs. 32, 33, 34) are highly refractile. They stain somewhat similarly to, but far from identically with the erythrocytes, varying from greenish to bluish or mauve with different pH of the Giemsa solution. They vary greatly in size, and sometimes occupy the greater part of the cytoplasm, which is then reduced to thin strands between a morula-like conglomerate of these inclusions. Russell-body plasma cells, when they occur, are very striking constituents of the Romanowsky stained smear, and would undoubtedly puzzle anyone not familiar with their appearance under these technical conditions.

Rarely, phagocytosed blood platelets may be seen in the plasma cells (figs. 35, 36).

Especially, perhaps exclusively, in plasma cells of the second type mentioned above, on rare occasions one may see large amounts of brilliant red (metachromatic) staining material incorporated in the basophilic cytoplasm, sometimes even preponderating over it; a peculiar "polychromatic" appearance may thus result (fig. 37). Such cells are not comparable with the so-called oxyphil plasma cell described by Dubreuil and Favre (1921) nor probably with the azurophil granule plasma cells mentioned by Naegeli (1923). Because of the metachromasia displayed towards the Romanowsky stain, one may suspect this material to be of mucoid nature. Morphologically it resembles the blue-staining material (supposed protein) in plasma cells described by Brass (1943b) and illustrated in his figure 2. Any statement regarding similarity of tinctorial properties is impossible, as this author used Weigert's fibrin stain.

Crystalline inclusions mentioned by Dubreuil and Favre we have never observed: Brass (1943a) emphasizes that they only occur under abnormal conditions (neoplasia). Neither did we see the intra-nuclear Russell bodies described by Apitz (1940) in plasmacytoma.

Plasma cells densely laden with haemosiderin may be encountered (fig. 38, 39), where intracellular haemosiderosis is a marked feature of the smear. Again this phenomenon appears mainly in the second type of plasma cell. We are inclined to think of this as being the result of haemosiderosis developed by the precursor cell, rather than haemophagocytosis by the plasma cell as such (cf. figs. 62, 63, with figs. 38, 39). The presence of large masses of haemosiderin in degenerated plasma cells is to us an indication that such haemosiderosis represents an unassimilable heirloom.

In this respect it is noteworthy that Klemperer (1923) found actively storing reticulum cells capable of "developing along hematic lines" (contra Maximow 1927c). It is further to be noted that Adamowicz (1930) found haemosiderin and non-iron containing pigment in plasma cells. He spoke of a rare and at times elective phagocytosis by plasma cells. Jaffé (1938) mentioned the presence of iron granules in plasma cells in the abdominal lymph nodes in cases of haemochromatosis. Alexeieff (1925a) stated that in ulcerative colitis 3-5 per cent. of plasma cells become phagocytic towards neutrophils, more rarely towards red cells; however they degenerate and cannot properly digest the phagocytosed elements. The plasma cell is regarded more as a toxinophage ("nephrophage") than a macrophage. Hertzog (1938) on the other hand could find no evidence of phagocytic activity, nor did Kiyono (1914b) observe any carmine hoarding by plasma cells. [For further opinions on this controversial point see Michels' (1931) review.]

The nuclei of plasma cells are usually rounded, but very striking departures may be seen (fig. 40). Not seldom plasma cells are binucleate (figs. 41 and 42). Multiple and lobed nuclei of these cells are apparently due to amitosis in many

cases (fig. 43), but plasma cells of reticulum cell and monoblastic derivation often have nuclear shapes (oval, kidney-shaped, crescentic, etc.,) probably depending on the same character of the cell from which they were transformed (fig. 40). Trinucleate and quadrinucleate cells may be encountered (figs. 44, 45), at aining such large size that it is quite possible that they arose as a change affecting reticulum cells which were already differentiating in the direction of megakaryocytes (i.e. megakaryoblasts) [15]. As in the uninucleate plasma cells, the presence of a centriole, or multiple centrioles, is often a prominent feature of these multinucleate cells (figs. 44, 45).

Ageing and degeneration are more clearly shown by plasma cells than by other elements found in spleen smears. This process has therefore had its fair share of attention in the literature (*vide* Michels 1931). It is in some ways very comparable with the transformation of an erythroblast to an erythrocyte. The nucleus becomes in turn more trachychromatic and pyknotic (fig. 46), and fragmented (figs. 47, 48); persistent nuclear fragments become rounded to form chromatic spherules, comparable with Jolly bodies, in an otherwise anuclear mass of cytoplasm (fig. 49). As a matter of fact, Erf and Herbut (1949) comment on the possibility, in sections, of confusing variants of plasma cells with haemocytoblasts, erythroblasts, and megakaryocytes.

The study of ageing phenomena in plasma cells inclines one to wonder whether any hard and fast line can be drawn between amitosis and karyorrhexis (see fig. 50). Indeed one has no certain knowledge that there is any essential difference of principle between these two processes. In other words karyorrhexis might—for all we know to the contrary—be correctly described as unequal amitotic division affecting a pyknotic nucleus. Both Maximow (1902), and Michels (1931), regard amitosis as peculiar to old, i.e. degenerating, plasma cells.

Certain phases of plasma cell karyorrhexis, in which the nuclear fragments are fine and numerous, form a quite important differential diagnosis from Koch's bodies of East Coast fever. (See fig. 48).

Mitosis is but rarely encountered (fig. 51). The plasma cells are obviously much more resistant than for example the lymphocytes to the effects of crushing and traction, and even in routinely prepared smears are often preserved intact. In poorly prepared or badly stained smears difficulty is sometimes experienced in distinguishing them from the red cell series (erythroblasts, normoblasts) encountered in the spleen in anaemias, or in very young animals.

The plasma cell affords a good example of the dynamic state of the cells constituting the blood-forming organs. Weidenreich (1909) even speaks of a transient, functional and irritative condition, and Downey (1911) holds similar views. In the light of recent developments in the field of histo-chemistry, the morphological characteristics of the plasma cell, more especially its cytoplasmic basophilia, gain new significance in terms of function.

Reference must be made to the demonstration of protein ribose nucleotides in the cytoplasm of plasma cells (however, mostly in immature cell types. A discrepancy thus remains to be explained if one wishes to link it unconditionally with cytoplasmic basophilia) by Bing, Fagraeus and Thorell (1945) using Caspersson's method, by Brenner (1946) using the methyl green-pyronin technique; and to the conclusions of Bing and Neel (1942), Bjørneboe, Gormsen and Lundquist (1947), and Fagraeus (1948) regarding the formation of antibodies by these cells. Dempsey and Wislocki (1946) have made similar suggestions concerning the lymphocyte. Under experimental attacks of this type one might hope to have the nature of Russell bodies and other inclusions elucidated.

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[15] Veratti (1905), believed the multinucleate plasma cell to arise as result of repeated nuclear division without cytoplasmic fission of pre-existent plasma cells.

4. *Reticulum Cells* (*Haemohistioblasts*) (Plate III, figs. 52-57), *and their Monoblastic Derivatives* (figs. 58, 59, 61, 64).

Under reticulum cells it is usual to include " undifferentiated " reticulum cells (associated with the production of reticulin fibrils) and phagocytic cells or fixed histiocytes. The distinction—which depends on the degree of phagocytic mobilisation—is largely artificial or subjective, and as is well known the " undifferentiated " or " resting " reticulum cell passes readily into the phagocytosing state.

These cells are not readily to be found in smears of normal spleen. Being in the form of a syncytial network, or because of their adherence to the reticular stroma, one may assume that they are not readily scraped from the cut surface of the organ, or alternatively, that they become damaged beyond recognition in the process. ([16]) Weil, Isch-Wall, and Perlès (1936) observed them only under abnormal conditions. [Their " cellules souches " or undifferentiated (stem) cells—no relation to haemohistioblasts was indicated—are undifferentiated " leucoblasts " and are similarly stated to be present only in certain pathological states (" leucoblastosis "), although we fail to see the difference between them (as illustrated) and so-called lymphoblasts. And their macrophages, " cellules à corps tingibles ", they derive solely from monocytes].

When seen intact (figs. 52, 53), they show spherical, beanshaped, or irregularly indented nuclei with a delicate nuclear membrane and extremely amblychromatic and leptochromatic nucleoplasm. Binucleate cells occur (fig. 53). In the one illustrated the extremely delicate and transparent appearance of the nuclei can be well appreciated. There are multiple pale bluish plasmasomes and " smoky " reddish karyosomes. The cytoplasm is irregular in outline, large in amount in more or less intact cells, but very pale staining—a light bluish grey. A distinct centrosome or diplosome is often to be seen (fig. 52).

The phagocytosing reticulum cells (histiocytes) are similar, but contain haemosiderin (fig. 54), or other phagocytosed inclusions (fig. 55 and 56) in the cytoplasm.

Both nucleus and cytoplasm may manifest morphological variations which presumably are the result of some additional physiological activity. The nucleus becomes " harder ", the chromatic material is thrown more sharply in relief and the cytoplasm increases in bulk and elaborates fine neutrophilic granules; vacuoles also occur (fig. 57). These changes could be interpreted as being due to evocation of the defence mechanism (vide Sabin, 1939). (Compare also our fig. 57, with that of Klemperer, 1938, p. 1636, fig. 27b). In this way the reticulum cell may " short-circuit " the development via monocytes to macrophages, and in this form it may appear in the blood stream in various pathological states (protozoal infections in our observations) as an overflow from the spleen and lymph nodes (see also page 99 for relationship of these cells to a certain type of sheath cell).

In view of Osgood's acceptance of Ringoen's (1927) explanation (Osgood and Ashworth, 1937), that cells like these appearing in the peripheral bloodstream under certain abnormal conditions and described by many authors as haemohistioblasts of Ferrata (see Rinehart, 1932), are really squashed and damaged elements, we must again draw attention to Maximow's (1926 and 1927c), and Ehrich's (1934) opinions that they are really activated and mobilized monocytes, or respectively, reticulum cells. Undifferentiated reticulum cells (haemohistioblasts *in sensu stricto*) also may occur in the peripheral circulation (e.g. Downey, 1938, Case IX, and plate II, fig. 26).

([16]) Reticulum cells are more readily studied in lymph gland smears, in which they have similar characters, but occur much more frequently. The existence of so-called " nucleo-cytoplasmic " territories (Hueck, 1920) probably allows some cells to be dislodged from the syncytium in more or less intact form.

All transitions (monoblasts) from reticulum cells to monocytes can be seen in spleen smears (figs. 58, 59, 60) ([17]), but under normal conditions, as will be mentioned again below, neither the monocytes nor their immediate precursors occur in large numbers. Some of these monoblasts may also contain haemosiderin (figs. 62 and 63) or other phagocytosed material (fig. 64).

5. *Monocytes* (Plate III, fig. 60).

In smears of normal spleens, cells identical with the blood monocytes are few in number (fig. 60). Textbooks are prone to give the impression that the spleen is an organ packed with monocytes, and further, that these are the essential phagocytic elements. This is not the case, in the bovine at any rate. As has been mentioned it is the lymphocytes which are the most important element in phagocytosis.

Moreover in spleen smears we see no evidence of transformation of lymphocytes into monocytes as has been suggested by Maximow (1927c) and by Bloom (1928a, 1938a), even in cases of monocytosis which we have studied. The transitions (monoblasts) are from the reticulum cells (see under this heading), (figs. 58, 59, 61).

This question has been dealt with, so far as general principles are concerned, under the heading " Problems of Cell Derivation ", and will be dealt with again in a note on the monocyte-macrophage problem (Chapter IV).

As has been mentioned, the reticulum cell as such, or in its stages of transformation to the monocyte, may transform into a type of plasma cell. Thus apparent transitions between monocytes and plasma cells may occur.

6. *Cells of the Schweigger-Seidel Sheaths* (Plate III, figs. 65, 66, 67, 68).

Our interest in the cells of the Schweigger-Seidel sheaths was originally stimulated not only by their morphological and physiological obscurity and the controversial nature of the literature, but by their especial interest to veterinary pathology. We refer to the selective localisation in these structures of the necrobiotic lesions of African Swine fever.

The identification of these cells was made possible by the comparative study of sections, isolated sheaths from pig spleens, and spleen smears.

In smears the sheath cells occur usually in clumps or groups (fig. 65). The endothelial cells of the arteriole may be intimately associated with them depending on the degree of disruption of the sheaths in making of the smear. In the routine type of preparation these cells frequently still lie in their proper cylindrical alignment, which then serves as a useful guide in identifying more or less intact sheaths by means of the low power objective.([18])

([17]) Often, when studying transitional types, we have been impressed by the discrepancy in the rate of differentiation of nucleus on the one hand, and that of the cytoplasm on the other, as far as such differentiation could be judged by purely morphological appearances. Figure 61 strikingly illustrates this point (see Legend). Bessis' (1946) and Bessis and Scebat's (1946) " loi d'asynchronisme d'évolution nucléo-cytoplasmique en cas d'hyperplasie " could be applied here, albeit in a somewhat wider sense. Needless to say, this leaves the confusion of classification worse confounded.

([18]) These sheaths must be distinguished from clumps of closely knit lymphoid tissue adhering to arteries (i.e. portions of Malpighian bodies), or from clumps of deficiently spread elements of the pulp adhering to parts of trabeculae.

For descriptive purposes we shall consider the sheaths as consisting of more centrally placed "fixed" cells, and peripherally situated more loosely arranged cells.

The central cells usually form the bulk of the sheath. They show great cohesion. Consequently the cytoplasm never appears intact: all that remains is odd wisps and strands with ragged edges. Intercellular boundaries cannot be discerned. The very intimate association with reticulin may be responsible for this. The reticulin may be so voluminous as to appear as a mass of pink-staining substance completely filling the space between the nuclei of the cell group (fig. 65).

The cytoplasm of sheath cells has a granular or muddy, opaque appearance and stains blue to reddish mauve. Various substances in amorphous, granular, globular or vacuolar form may be seen, either alone, together, or in varying combinations. A detailed description will be given below where the peripheral cells are dealt with. These substances are poorly or not at all developed in the central cells, hence the cytoplasm of the latter is clearer, at least where it is not obscured by reticulin. The centriole, sometimes seen as a double granule or rodlet, is very prominent and distinct (fig. 65).

The nucleus varies from roughly rounded to elongate forms. A dozen random measurements in smears from two different animals varied from $8 \cdot 5 \times 7 \cdot 8\mu$ to $15 \cdot 6 \times 7 \cdot 0\mu$. The nuclei exhibit a striking angularity of outline—roughly quadrangular, polygonal, rectangular, and triangular or turnip-shaped forms predominate, but irregularly oval, elongated or lobated types may also be seen. Irregularity of contour is a characteristic feature: wrinkling, bulging, indentation, and deep folding of the nuclear membrane. Comparison with sheath cells as seen in well prepared sections, shows that these irregularities are not mechanical artefacts.

The nuclear texture is characterised by hardness of appearance. There are two to five heavily staining chromatin knots of irregular shape. The intervening areas are more leptochromatic, yet trachychromatic. No nucleoli are visible—(this does not necessarily imply that they are absent). The uneven nuclear membrane is very thick and hyperchromatic. The smaller and more rounded the nucleus the more pachychromatic and trachychromatic does it appear.[19]

The peripheral cells lie scattered around the circumference of the mass formed by the body of central cells. There may only be a few present, but (presumably when the sheath has been stimulated to greater activity) they may form a more conspicuous part of the ellipsoid. As they are not so closely bound together as the central cells, they reveal a distinctly outlined, more voluminous cytoplasmic body, varying from 20–25μ by 15—20μ in size. They usually have a rectangular or elongated polygonal shape. Details of the cytoplasmic contents already mentioned previously are as follow:—

(1) Amorphous basophilic material, rather poorly developed (fig. 66), and varying in fineness.

(2) Fine reddish brown neutrophilic granules (figs. 66 and 67).

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[19] If staining and mode of preparation are not favourable, the nuclei of sheath cells may be mistaken for those of lymphocytes and *vice versa*. Under similar conditions one may confuse elongated sheath cell nuclei with endothelial nuclei or with sinus cell nuclei (q.v.). It must be emphasised that preparations which at first sight appear to have been well-stained often reveal most misleading similarities of nuclear texture between unrelated cell types.

(3) Larger granules, in parts so closely resembling the chromomeres of blood platelets, that their possible identification with the latter should be seriously considered (fig. 68). These granules are the least frequently observed.

(4) One or more rounded or irregular vacuoles, more or less heavily outlined, and containing what appears to be platelet material in varying stages of disintegration (fig. 66). This platelet-like material may also be intranuclear in position (fig. 67).

(5) Some clear vacuoles, which could represent the final stage of dissolution of such platelets.

(6) One or more brown- to brownish-mauve-staining globules, varying from about $0 \cdot 5\mu$ to $3 \cdot 0\mu$, are fairly frequently observed (fig. 67). We have been able to observe gradations in staining reaction of these globules from pale brownish-mauve to the intense purple of chromatin, and we therefore strongly suspect this substance to be of chromatinic derivation.

The centriole is distinct (figs. 66 and 67) although not quite so prominent as in the central cells.

Free sheath cell cytoplasm, characterised by the contents described above, occurs as rounded plates or large irregular sheets.[20]

The nuclei of the peripheral cells tend to be more regular in outline than those of central cells; they also reveal a more open and regular grain, with lighter staining centres in the chromatin knots (fig. 67).

From these studies, albeit purely morphological, it is feasible to consider that sheath cells gradually undergo a process of mobilisation, which is expressed by rounding up of cytoplasm, by the nucleus becoming more vesicular and regular in outline, and more even in texture, and by narrowing of the nucleo-cytoplasmic ratio. This process occurs at the periphery of the sheath. These mobilised cells may then exhibit phagocytosing properties.

When we compared this peripheral sheath cell type with the activated reticulum cell, as depicted in fig. 57, we were impressed by their similarity. In sections of a well-perfused spleen of a bovine suffering from a combined infection of various protozoan parasites (*Babesia*, *Theileria*, and *Anaplasma*), we were unable satisfactorily to demonstrate well-formed Schweigger-Seidel sheaths and in smears no sheath cells of central type could be found. In both sections and smears, mobilised cells (i.e. peripheral cells of the type shown in fig. 67, and activated reticulum cells—see fig. 57) dominated the picture. This strongly indicated a state of complete mobilisation of the Schweigger-Seidel sheaths. That both reticulum cells and sheath cells are able to undergo a mobilisation to produce an exactly similar type of cell, was shown by finding such cells in lymph node smears obtained by puncture of the prescapular node of bovines at certain stages of reaction to artificial East Coast fever infection. Here the presence of mobilised sheath cells could of course be excluded.

These observations lend further support to the thesis that sheath cells and reticulum cells are physiologically closely allied, if not identical. Not only in their activity in defence reactions, but also in the production of reticulin are the sheath cells to be regarded as nothing but morphological variants of the reticulum cell, and

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[20] If, as we believe, peripheral sheath cells and mobilised reticulum cells are morphologically identical, then it becomes impossible to distinguish between mobilised reticulum cell and peripheral sheath cell cytoplasm.

the fact that they are very closely packed together at certain strategic points of the vascular system of the spleen, may conceivably be the sole reason for their different appearance in the resting phase.

These observations and conclusions will be discussed later in the light of the recent literature. (See page 122, Chapter IV sub-heading 4).

7. *Endothelial Cells* (Plate IV, fig. 69).

Here we refer to the common endothelium or vascular lining cell, in spleen smears probably derived exclusively from the arterial capillary or capillary arteriolar vessels—not from the venous sinuses. They are not numerous. Their appearance in Giemsa-stained smears will be familiar to those who have examined intima smears for the diagnosis of *Rickettsia ruminantium* infection. They are most certainly recognized when retaining their continuity, i.e. in the form of a tube within which the contained blood cells may still be present (fig. 69). The nuclei are oval, but not seldom become extremely elongated through the tension set in drawing the smear; they may then stain more deeply than usual and it is possible in these circumstances to confuse them with smooth muscle cells. Apart from usually being of narrower and longer dimensions, and tending towards a more pointed oval shape, the nuclei in most respects—nuclear membrane, texture, nucleoli—appear very similar to those of reticulum cells. However, they are slightly less leptochromatic and the true nucleoli are less distinct. The cytoplasm is weakly basophilic, and is homogeneous, or under certain conditions of favourable technique may show numerous minute vacuoles, sometimes also faint neutrophilic granules almost at the limit of visibility, as well as fewer, somewhat coarser, pale blue granules. A moderately prominent centriole may be seen. The cytoplasmic characters of endothelial cells may be obscured by reticulin fibres with which they are intimately associated, (see description of these fibres).

8. *Sinus-Lining Cells* (commonly termed " Littoral Histiocytes ", " Reticulo-endothelial cells ") (Plate IV, figs. 70 and 71).

The bovine spleen contains graded sizes of venous sinuses emptying into the general venous system and varying from capillary to venous dimensions. These sinuses are morphologically identical with the intralobular veins, the difference being merely one of size, (see also Foot, 1927; MacKenzie, Whipple and Wintersteiner, 1941). *They are all lined by identical cells which present distinct differences from either endothelial cells or reticulum cells.*

There is a common impression that these cells are essentially identical with reticulum cells, the difference being merely their topographical relationship to sinusoids and enhanced powers of phagocytosis, (the latter perhaps merely depending upon their superior opportunities).

This view is implicit, and has often been quite definitely stated, in various writings on the " reticulo-endothelial " concept. One of us previously subscribed to it (Jackson, 1934). In view of such prevalent impressions, we wish to state at once: (*a*) *that the sinus cells of the bovine spleen are morphologically quite distinct from reticulum cells (as well as from endothelial cells);* (*b*), *that we have never seen any evidence of phagocytosis by these cells—either under normal or under pathological conditions—including those in which the reticulum cells showed great phagocytic mobilisation,* (*c*) *that we have likewise seen no evidence of their transformation into monocytes or any other cell type.*[21]

[21] In bloodsmears, we have seen desquamated cells of the type to be described. The fact that they were readily recognisable serves to emphasise our doubt that they were turning into any other kind of cell.

The sinus cell (fig. 70), has a much more trachychromatic nucleus and a heavier nuclear membrane than has the reticulum cell or the endothelial cell. *The nuclear pattern is characteristic and can scarcely be confused by the experienced observer with that of any other cell of the spleen* (with the possible exception of some of the Schweigger-Seidel sheath nuclei). It is composed of large angular blocks of chromatin separated by a system of narrow pale-stained lines (" nuclear sap "): a crocodile-leather appearance. No nucleoli are seen. The cytoplasm is prolonged into the tapering poles of a spindle and in smears usually preserves a gently curved or sickle-shaped outline. It contains numerous minute neutrophilic rodlets (fig. 71), or, when not so well fixed, may present a diffuse pink coloration, (fig. 70). In addition, quite constantly, the cytoplasm contains coarse angular pigment particles, staining with Giemsa a Prussian blue colour slightly tinged with green. These are situated in one or two clusters related to the poles of the nucleus. This is apparenly the " haemofuscin " pigment of Mallory.[22] It is a deeply basophilic substance and thus stains strongly with Giemsa, methylene blue, basic fuchsin, etc. It fails to give the Prussian blue reaction with potassium ferrocyanide after hydrolysis with hydrochloric acid at room temperature (the usual method of performing this test)—a circumstance which has given rise to the widely accepted view that it is an iron-free substance. However, if the hydrolysis is assisted by heat, it does give the Prussian blue reaction.[23] There is an entire absence of evidence that this iron-containing pigment is derived from haemoglobin [24] and, as stated, we have never observed these cells to phagocytose erythrocytes.

Although these cells have received their due share of attention in the literature (*vide* Klemperer, 1938), their appearance in smears has been remarked upon briefly only by Schmaus and Herxheimer (1910), by Naegeli (1928), and by Foot (1927). Schmaus and Herxheimer state: " An Zupfpräparaten fällen häufig besonders in Fällen von akutem Milztumor, schmale sichelförmige Elemente auf, welche den . . . Venen-endothelien entsprechen." Naegeli differentiates their nuclei from those of lymphocytes, and describes " charakterische spärliche azurophile Granulation " in the cytoplasm with Giemsa staining, and fine iron-containing granules, the latter only present in disease. The illustration does not allow of critical comparison with our neutrophilic rodlets and pigment granules. Foot's illustration was mainly for the purpose of showing alternating light and dark cytoplasmic areas, which produce a cross-striated appearance in sections. These, as well as endothelial ridges. basal plates and cytoplasmic notches as described for the human and some animals (dog), are absent in the bovine (cf. also Klemperer 1938, figs. 24 and 25). We have not observed the characteristic " chromatin bar " in sinus cell nuclei as mentioned by the latter author.

The cells seen in spleen cultures, and named " endothelial cells " or " sinus-endothelials " (Fazzari, 1926; Erdmann, Eisner and Laser, 1926) are difficult to evaluate, because of lack of topography and of the pronounced morphological alterations which cells generally may undergo in culture—difficulties which these authors themselves stress. The cell type described and figured by Fazzari as " endothelial cell " from the spleen of the chick embryo does somewhat resemble our description, not that from the adult mouse, however. On these grounds one cannot accept the statement that transformations between the endothelial cell and the reticulum cell occur. We have seen nothing to indicate this in our material.

9. *Smooth Muscle Cells* (Plate IV, figs. 72, 73).

In the bovine spleen (as can be seen in sections) the muscular trabeculae ultimately branch into fine terminal chains of smooth muscle cells, coursing

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[22] and the " cytosiderin " of Gillman and Gillman (1945). Risel (cit. Pick, 1925) finds " das Protoplasma dieser Zellen (endothelial cells of venous sinuses) meist ganz dicht bestäubt mit bräunlichen körnigem Pigment, das zum Teil die Eisenreaktion gibt " in cases of Morbus Gaucher. No description of the normal state is given.

[23] We are indebted to the Department of Microscopic Anatomy, Medical School, Johannesburg, for this valuable hint.

[24] Cf. the occurrence of the same material in liver cells, adrenal cells, etc.

intimately through the pulp, apparently affording support to the venous sinuses and also apparently becoming continuous with the reticular syncytium. Thus, as would be expected, muscle cells are fairly numerous in the bovine spleen smear. We have seen as many as ten in a single oil-immersion field.[25] They are well preserved in ordinary smears. The nuclei are cigar-shaped, with somewhat square ends, darkly staining, coarsely textured, and show no nucleoli. The cytoplasm is a very elongated spindle, staining rather brightly basophilic and having a characteristically refractile and opaque appearance. Especially associated with the edges of these cells in bundles, reticulin fibres may be identified (fig. 73).

Smooth muscle cells, especially as regards their cytoplasm, are relatively very resistant to the effects of decomposition, and in decomposed smears are often the sole remaining recognisable elements.

10. *Serosal Cells* (*Mesothelial Cells*) (Plate IV, figs. 74-77).

For many years we had noted and puzzled over the occurrence of cells of "epithelial" appearance in routine spleen smears (fig. 74). They occur quite inconstantly and in the majority of smears will not be seen in the time available for routine examination; in a minority of smears one, or few, may be found on searching. But occasionally one encounters (from the field) smears in which these cells are present in striking numbers, in rare instances predominating over all other elements.

On account of their morphology, these cells contrast strongly with all other elements of the spleen smear. They may occur singly or in small or larger groups or sheets. When not distorted they have a distinctly polygonal or angular outline. The nuclei are highly characteristic and epithelial-like: leptochromatic, yet trachychromatic, composed of innumerable sharply defined small angular blocks of chromatin. The nuclear membrane is very poorly developed; indeed a very usual appearance is that the peripheral chromioles appear as if in direct contact with the cytoplasm, so that the edge of the nucleus presents a ragged appearance. The nuclei vary from ovoid to elongate, but the formation of ring-shaped nuclei (fig. 77) is often a striking feature.[26] All stages of this transformation can sometimes be followed: bean-shaped nuclei, crescents (fig. 76), fusion of the horns of the crescent to produce a closed doughnut-like form enclosing a central area of cytoplasm (figs. 74 and 77).

The cytoplasm of these cells is scanty to moderate in volume, is granular and stains from light bluish-grey to pale blue. (The more basophilic hue depicted in figs. 76 and 77, is due to more intense staining of the preparation.) A narrow, incomplete perinuclear halo is often visible. Under unusually favourable conditions one can differentiate a regular outer seam of more acidophilic homogeneous exoplasm from the main mass of greyish-blue endoplasm (fig. 75). This outer seam could possibly have some relationship to the outer plate described by Kolossow (1893), but in view of the differences in method of study, no definite statement is possible.

No acidophilic area related to the indentation of the nucleus (where this is present) was apparent in smears (cf. Maximow, 1927a), nor did we note the presence of any fatty droplets (cf. Cunningham, 1922). That the latter may be absent was indicated by Maximow

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[25] This may represent hyperplasia of these elements, or alternatively, the presence of excessive trabecular material in the smears perhaps on account of autolysis; usually they are far less numerous.

[26] It is surprising that no mention of ring-shaped nuclei is to be found in studies of the peritoneum based upon the examination of sections.

(1927a). (According to Sabin, Doan and Cunningham, 1925, these droplets only develop after desquamation). In smears, cilia were never observed by us, nor evidence of syncytium formation, nor of the presence of intercellular bridges (cf. Cunningham. 1926, and authors cited by him).

Centrioles are often distinct, sometimes prominent.

The presence of these elements in spleen smears depends on scraping against the serosa when making the smear, or on carrying serosal cells over the cut surface with the knife when incising the organ. Preponderance of mesothelial cells over other elements in occasional routine smears is due to drawing a slide over the incised spleen held in such a manner that the edges are inverted. The identity of these cells was established by comparison with smears deliberately made from the splenic serosa (or from any other part of the peritoneum) and by making control smears in which, by picking out pulp from the centre of the organ, contact with the serosa was avoided. As the appearance of mesothelial cells in smear preparations was unknown, it will be appreciated that the only certain means of establishing the identity of these cells was by direct comparison with known mesothelial cells.

It may be mentioned that under certain conditions of technique which we regard as inferior, or in badly prepared smears, the nuclei of serosal cells may appear far more amblychromatic and still more leptochromatic than described and illustrated. It is quite possible to confuse the cells with reticulum cells under such conditions. Here the occurrence in sheets and angular outlines is helpful, as well as the presence of ring-shaped nuclei, when they occur.

11. *Mast Cells* (Plate V, figs. 78 and 79).

Mast cells are sometimes present in extraordinary numbers in spleen smears of clinically healthy cattle.

Comparison with sections indicates their extravascular situation, i.e. they are " tissue basophils ", not " blood basophils ". They possess simple oval nuclei. One would expect to encounter occasional blood basophils from the blood contained in the spleen. However, if this is so, we had no means of, or interest in, distinguishing such elements from tissue mast cells. The local formation of mast cells was further confirmed by examining foetal spleen smears in which the stages of their formation can be clearly followed.

The granules of the mast cells will be referred to later.

12. *Eosinophils* (Plate V, figs. 80, and 81).

Eosinophils often by far outnumber neutrophils in smears from clinically healthy animals.

This may indicate any one of three possibilities: (1) selective stasis of these elements in the vessels of the spleen, and/or emigration into the pulp; (2) local production in the spleen pulp in adult life; (3) cells which failed to emigrate into the blood after having been produced in the spleen in embryonic life.

We do not propose to go fully into these questions here. No cells which one could possibly term eosinophil pro-myelocytes were seen in spleen smears of adult animals. Yet the possibility remains that, if the splenic eosinophils are locally produced, their precursors might be cells not of myeloblastic type (but e.g. of medium lymphocyte type).

We have observed two very typical features of these splenic eosinophils. Firstly, the characteristic occurrence of coarse refractile granules quite different from the specific granules, up to about half a dozen in number (fig. 81). They change from black to luminescent as one focusses them. They were described for human eosinophils as α^1 granules by Liebreich (1916), who considered at length numerous theories as to their origin, and concluded that they are probably of nuclear origin and consist of " nuclein ". We do not agree. These granules need more investigation. They may conceivably be nothing but centrioles, a possibility which did not occur to this author. In bloodsmears of the ox they appear far less conspicuous than in spleen smears, and it is possible that their greater prominence in splenic eosinophils is a sign of youth.

Another common feature of the splenic eosinophils is the deficient filling of the cell with granules, so that considerable areas of clear basophilic cytoplasm are seen. This phenomenon is regarded by Naegeli (1923) as definitely indicative of immaturity. The splenic eosinophils are often far more " stabkernig " than the blood eosinophils, i.e. the nuclei tend to be simple or bilobed, not horse-shoe shaped or sinuous. Neither of the last two mentioned appearances is constant.

The implications cannot be fully dealt with here. It must be sufficient to quote a few findings and opinions, and to refer the reader to Klemperer (1938, pp. 1649-50) for a fuller discussion.

Svensson (1936) often found eosinophils in the human spleen, even in apparently clinically healthy subjects, but never observed mitoses or myeloid cells. He regards the eosinophils as being of bone marrow origin but attracted to the spleen by chemotaxis (an opinion only slightly different from the first possibility stated above).

Bertelsen (1938) in a rather unconvincing article described the presence of eosinophil myelocytes occurring without exception in apparently clinically healthy subjects. In addition he found neutrophil and basophil myelocytes. He expressed himself in favour of a local origin, probably from large lymphocytes or from reticulum cells. Lewin (1929) saw immature forms of eosinophils in the spleens of human foetuses and of newborn babies. Paremusoff (1911) pictured eosinophil myelocytes (termed by him pro-myelocytes) in smears from the guinea-pig spleen.

Bock (1932) believes the spleen to promote granulocyte maturation, but did not observe unripe myeloid cells. Biggart (1932) finds no evidence of production of eosinophils in the spleen, and no increase of the eosinophil content of the spleen in cases of general eosinophilia as compared with the increase in the circulation.

It is quite conceivable that a de-differentiation of the eosinophils may occur in the spleen, as Richter (1942) described for neutrophils in oxalated or citrated blood *in vitro*.

Phagocytic activity of eosinophils, as reported by Weinberg and Séguin (1915) towards bacilli, protozoa, and erythrocytes, and by Hertzog (1938) and by Strumia and Boerner (1937) towards bacteria, has not been observed by us. Nattan-Larrier and Parvu (1909) as well as Achard, Raymond and Foix (1909) have reported on phagocytosis by eosinophils, the latter finding the phagocytic activity normally to be very feeble, and only enhanced in pleural exudate, whereas the former authors found it to be quite pronounced but always less than that of neutrophils.

13. *Neutrophils* (Plate V, figs. 82 and 83).

Neutrophils are sometimes no more numerous than would be explicable on the basis of the blood content[27] of the spleen; in other cases they are present in much larger numbers, and sections may reveal a concentration of these elements in the sinuses. As discussed in the preceding section one cannot assume that this indicates merely a stasis of (myelogenous) neutrophils.

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[27] The reader may be reminded of the low percentage of these cells in bovine blood.

The neutrophil granulations of bovines usually stain somewhat poorly with ordinary Giemsa or Pappenheim technique, but in the splenic neutrophils it is virtually impossible to stain them satisfactorily without modifying these techniques. Further we have the impression that the splenic neutrophils tend to possess less polymorphic and more lightly staining nuclei than the average blood neutrophil. Here again it is possible that one is encountering signs of immaturity. Certainly the evidence is far from indicating any selective holding up of aged neutrophils by the spleen.[28]

We have seen spleen smears in which many of the neutrophils contained recently phagocytosed erythrocytes (fig. 83). It is quite likely that these smears did not originate from clinically healthy subjects.

Jochmann and Blühdorn (1911) have described "polynuclear leucocytes" containing red cells in the blood in cases of acute myeloblastic leucaemia. This phagocytosis they presume to occur in the spleen, as it was more evident in splenic puncture preparations than in the peripheral blood. These authors cite Pappenheim (1908) and Wechselmann and Hirschfeld as having observed a similar phenomenon. Hirschfeld (1907) is also cited as stating that there is marked erythrophagocytosis by "leucocytes" (presumably neutrophils) after potassium chlorate, or toluylene-diamine or pyrogallic acid dosage. Hirschfeld and Sumi (1925) have described erythrophagocytosis by neutrophils in the peripheral circulation in rats after splenectomy or after intraperitoneal injections of blood. The neutrophil was, however, the least common phagocytic element as compared with "monocytes" and "endothelials". In the bone marrow in cases of pernicious anaemia, Doan (1926) noted occasional erythrophagocytosis by polymorphs—nucleated macrocytes, and microcytes of mature type, being involved.

Neutrophils may also contain phagocytosed blood platelets (fig. 82). Taliaferro and Klüver (1940) have observed phagocytosis of platelets and of small clumps of malarial pigment in neutrophils in the peripheral blood of Panamanian monkeys after infection with malaria (*P. brasilianum*). Erythrophagocytosis was never seen by them.

The described activities of neutrophils would seem to be in contrast with the classical conception of the phagocytic abilities of these elements, and would seem to cut across Metchnikoff's original distinction of "microphages" from "macrophages".

14. *Megakaryocytes* (*Plate V, fig.* 84).

These are encountered in smears from young subjects. They are few in number and identical in morphology with those of bone marrow, i.e. giant cells with much lobed nuclei and cytoplasm packed with groups of fine azurophil granules, the blood platelet chromomeres. Quite usually, as in bone marrow, these cells contain what have been described in the literature as *phagocytosed* leucocytes, especially neutrophils. We are however strongly inclined to believe that the truth of this matter is exactly the opposite of this conception, i.e. that it is the megakaryocyte which is the victim and the leucocyte the agressor; and that where leucocytes have to deal with elements too large to be phagocytosed *in toto*, they actively penetrate into them and as it were accomplish "phagocytosis from within". (Compare the neutrophils to be found within cornified epithelial squames in vaginal smears and the remarks of Cowdry, 1938, apropos the observations of Stockard, 1932, on what might be termed "endophagocytosis"). Downey, Palmer, and Powell (1930) preferred to leave this question open.

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[28] Weil *et al.* (1936) indicate the presence of myelocytes and metamyelocytes in "normal" human spleens (obtained from cases of purpura). This we did not observe. It is important, however, to bear in mind the phenomenon of de-differentiation with confluence of nuclear segments which neutrophils may undergo under abnormal conditions (i.e. in stagnant blood). (See Richter, 1942, and authors cited by him).

In the bone marrow of essential thrombocytopenia Schminke (1930) described " Anschmiegung " (i.e. attack from without) by *lymphocytes* against megakaryocytes showing granule-free cytoplasm and degenerating nucleus. This he regards as phagocytosis of these giant cells.

15. *Erythrocytes* (Plate V, fig. 85).

These need no description. The staining, as is well known, varies from yellow through all intermediate shades to blue, depending on the pH conditions of the technique. In routine smears, as will be mentioned, they may be considerably simulated by what are probably contaminating spores of fungi and also by globular fragments of cytoplasm freed as an artefact during the drawing of the smear.

Normoblasts, as described in the supposedly normal human spleen by Weil and co-workers, we have never seen in healthy adult bovines.

16. *Blood platelets* (Plate V, fig. 86).

These need no special description. Superimposition on cells is to be distinguished from thrombophagocytosis. Platelet clumps can be distinguished from, but might easily be confused with, masses of cytoplasm torn from megakaryocytes (see below). *Occasionally the chromomeres of platelets are so coarse as to cause confusion with the so-called free Koch's bodies of East Coast fever, even to fairly experienced examiners.* The phagocytosis of platelets has already been described; it will be discussed later.

17. *Connective Tissue Fibres* (Plate V, figs. 87, 88, 89, 90).

The connective tissue fibres of commonest occurrence in spleen smears are reticulin fibres (fig. 87). With Giemsa technique they are poorly stained and extremely inconspicuous structures. They often become considerably blurred in the making of the smear and appear as a pale rose-pink delicate amorphous mass. They are especially to be recognised when associated with vascular endothelium, groups of Schweigger-Seidel sheath cells (plate III, fig. 65) and bundles of smooth muscle cells (plate IV, fig. 73) but may appear isolated, as in figure 87.

As the appearance of reticulin in Giemsa stained smears was quite unknown to us, the identification was based on staining control smears with a modified Bielchowsky silver technique. *Reticulin stains with Giemsa much more lightly than the chromatin of nuclear smudges* (*see plate VI, figs.* 107, 108, 111), *with which it should not be confused.*

Elastic fibres (figs. 88 and 89) would similarly probably not be recognised in smears unless attention were drawn to them. They are characterised by their hard outlines and refractile appearance, and are virtually unstained. They vary greatly in thickness and tend to be U- or J-shaped, or to occur in coils.

Collagen (fig. 90) is very seldom seen as almost unstained wavy bundles composed of numerous fibrils.

18. *Pigments* (Plate V, figs. 91 and 92).

(i) *Haemosiderin:* The most striking pigment in spleen smears is haemosiderin (fig. 91). *It very often occurs in huge quantities in preparations from clinically healthy subjects* (which however have quite probably at some previous time passed through an attack of piroplasmosis, etc.).

It is recognised as granules or flakes of varying size, staining a characteristic olive-green colour (due to superimposition of the basic component of the Giemsa stain on the original golden colour of the pigment). This colour may vary from yellow to dark brownish-green, depending on the intensity of staining of the smear. In most smears the overwhelming number of these granules appear to lie free among the cellular elements, and this does not appear entirely to be a mechanical or displacement artefact: apparently free haemosiderin may also be identified in spleen sections. This extracellular appearance of haemosiderin has also been noted by a number of others (e.g. Spadolini, 1928, who supposed that it was set free on disintegration of the phagocytes).

Intracellular haemosiderin (plate I, figs. 20, 21; plate II, figs. 38, 39; plate III, figs. 54, 62, 63) in phagocytic cells is recognised by the same characters, but usually occurs in much smaller quantity. A sender of smears made from haemosiderotic spleens should not be blamed for having submitted " dirty " preparations. Occasionally one may experience difficulty in distinguishing haemosiderin from extraneous dirt, especially in over-stained smears. If distinction is necessary. recourse can always be had to the Prussian blue reaction.

(ii) "*Haemofuscin*": (Fig. 92). The appearance of this pigment has been described when dealing with the sinus cells, with which it is characteristically associated (plate IV, figs. 70 and 71 and page 101.) Large quantities of apparently free " haemofuscin " are often seen in field smears purporting to be spleen smears. but these are invariably lymph-gland smears, of which this pigment is a much more striking component. The deeply staining " haemofuscin " must not be confused with contaminating coccoid organisms (cf. plate VII, fig. 123), coarse stain deposit, or free basophil granules (cf. plate VII, fig. 116).

CHAPTER III.—THE ARTEFACTS OF THE SPLEEN SMEAR.

As already mentioned and discussed, the routine spleen smear is a mass of confusing appearances, the great majority of which are not analysed or recognised for what they are. Because of the impossibility of distinguishing between the various types of artefacts and some pre-existing artefact-like structures (see pp. 82-85 all these appearances will be described under the heading of "artefacts".

A. *Mechanical Artefacts (Distortion, Displacement, and Crushing and Tearing Artefacts).*

1. *Distortion of the Cell.*

The process of smearing involves the " pancaking " of spherical cells to circular discs and sometimes produces the appearance of thickened edges; this may involve the nucleus if it lies near the edge of the cell (plate I, fig. 10). It is mostly seen when light pressure has been exerted in making the smear, and is thus an embarrassing factor only in the better type of smear. Occasionally one may see a peculiar pseudopodium-like projection of an otherwise spherical cell. especially when the cytoplasm is very voluminous (plate I, fig. 9), but it is not possible definitely to state whether this is an artefact. It may represent the " budding off " of cytoplasm according to the interpretation of Dougherty and White, (1945).

2. *Cytoplasmic Fragments* (Plate VI, figs. 93-100).

As has been explained, these are exceedingly numerous in routine smears. They are mostly derived from the fragile lymphocytes. When torn from the cells these pieces of cytoplasm tend to round up into discrete spherical bodies (fig. 93).

Like the lymphocytes, from which they are mostly derived, they may contain unstained vacuoles (fig. 94), azur granules (fig. 95), phagocytosed erythrocytes (fig. 96), phagocytosed platelets (figs. 94, 97 and 98), etc. Even centrosomes are sometimes identifiable in free cytoplasmic fragments (fig. 98). Fragments of plasma cell cytoplasm may contain vacuoles or Russell bodies (fig. 99.) *The cytoplasmic fragments of lymphocytes play an important part in routine diagnosis which does not seem to be generally understood:*—When derived from *Theileria parva*-infected lymphocytes they are spoken of as "free" Koch's bodies. But we are strongly inclined to believe that these bodies are always intracellular, that the distinction between intracellular and "free" Koch's bodies depends merely on mechanical displacements, and that the proportion of "free" Koch's bodies is thus in inverse proportion to the degree of refinement of the smear technique.

Fragments of cytoplasm, when about the size of red cells, may fairly easily be mistaken for anisocytes or polychromatic poikilocytes (cf. plate V, fig. 85 with plate VI, fig. 93).

Fragments containing coarse azur granules or phagocytosed platelets with coarse chromomeres form perhaps the most important source of confusion with the "free" Koch's bodies of East Coast fever (see figs. 95, 97 *and* 98). *This is indeed the* bête noire *of routine examination for East Coast fever, especially in badly prepared smears.*

Cytoplasmic fragments from other types of cells can often be recognised by their characters. Those derived from megakaryocytes (fig. 100) could of course fairly easily be confused with masses of blood platelets. (Note however, the two diplosomes visible in this case).

In addition, routine smears almost invariably show a light grey to blue, finely or more coarsely granular background, very picturesquely described by Weil et al. (1936) as " . . . un fond plus ou moins homogène, plus ou moins granuleux . . . semblable à un tapis troué et taché . . ." This represents either the effect obtained by effacing of cell boundaries and squashing of cytoplasm *in situ*, with the additional admixing of plasma, and the coagulative and denaturing effect of fixation; or it indicates a further degree of damage to the globules of cytoplasm, previously dislocated from the nucleus and now finely ground up.

3. *Nuclear Artefacts* (Plate VI, figs. 101-112 and plate VII, fig. 113).

In most smears, as has been pointed out, the appearance of "naked" nuclei, especially of the lymphocytes, is produced through the effects of pressure and traction (figs. 101 and 102). The nuclei may actually be completely denuded, or wisps of cytoplasm may be found clinging to them (fig. 103), or the naked appearance may be a false impression produced by effacing of cell boundaries and damaging of the cytoplasm, so that it merges in the general background of diffusely spread cytoplasmic material described above.

The three sizes of nuclei belonging to the three types of lymphocytes can be recognised (figs. 101, 102, 103). Naked nuclei of reticulum cells (fig. 104) are also characteristic, while those of megakaryocytes are of course unmistakable (fig. 105). The "sinus cell" nucleus very frequently has haemofuscin particles adherent to it, often in various displaced positions (e.g. over the nucleus) (fig. 106). These, when present, serve as an excellent feature for identification, quite apart from the texture of the nucleus.

In the average smear many of the nuclei are reduced to smudges (figs. 107, 108), produced by excessive pressure. *Superimposition of a naked nucleus on a*

nuclear smudge—a sort of "cell" artificially manufactured during making of the smear—is not uncommon (fig. 109) and can lead to puzzling appearances. The smudge is then mistaken for chromatic or basophilic cytoplasm belonging to the nucleus. *This appearance has been featured by Hargraves, Richmond, and Morton* (1948) *and described as one type of "tart cell"* (their figs. 1a and 1b) in their bone marrow preparations. The significance of this appearance was not understood by these authors, who moreover obtained no enligh enment from "numerous visiting haematologists", nor from the members of the Haematology Club in Chicago. Another type of their "tart cell" (their fig. 1c) is probably dependent on artificial juxtaposition of nuclei with slight smudging of the one nuclear moiety. Such misconceptions led us at that time to feel some apprehension about the so-called "L.E." (lupus erythematosus) cell described by these authors, but the genuine nature of the latter seems now to have been established.

Traction results in the drawing out of filaments of the highly ductile chromatin (fig. 110). *Many routine smears are composed predominantly of a tangled and confused mass of these chromatin filaments* (fig. 111), which of course stain identically with chromatin, i.e. much more deeply than any variety of connective tissue fibres. When single filaments snap off, they retract and recoil. They are then even more intensely chromatic and *may resemble chains of Anthrax bacilli or contaminating organisms* (fig. 112). In shape, but never in staining reaction, they often resemble elastic fibres.

Rounded globules of nucleoplasm are sometimes scattered about at odd intervals, and could be confused with cocci, were it not for their irregular size. Rarely a whole smear is dotted with these variously sized bodies (fig. 113). Besides traumatic forces during preparation of the smear, autolysis and decomposition are probably responsible for this.

4. *Free Cytoplasmic Constituents* (Plate VII, figs. 114-122).

Granules freed by the rupture of eosinophils during the drawing of the smear should be readily recognisable and are often present in large numbers. In shape the eosinophil granules are short rods, but when less well fixed they may appear rounded. They stain characteristically (bright yellowish red to red) and are refractile. *When superimposed on erythrocytes they may be a cause of confusion or at least annoyance in routine examination* (fig. 114). When such erythrocytes are stained bluish due to pH conditions, puzzling appearances can result (fig. 115).

Free mast cell granules (fig. 116) are often seen in surprisingly large numbers; some smears are full of them. *When superimposed on erythrocytes* (fig. 117) *they may be confused with Jolly bodies,* as they may appear spherical with a lighter centre on focussing. Others however, are short rods and these *when superimposed on red cells are difficult to distinguish from anaplasms* (figs. 117 and 118). Basophil granules should be distinguished from extraneous material, such as stain deposit (fig. 128), or contaminating cocci (fig. 123). Cocci are larger and also more regularly spherical in shape, but stain very similarly. Like free eosinophil granules, free basophil granules are an annoyance in routine smears, because, al hough usually there is no difficulty in dismissing them as non-pathological, they attract the eye during examination.

Free Russell bodies (fig. 119) either singly or in groups (fig. 99), are sometimes encountered. They are recognised by their spherical outline, highly refractile nature and greenish to bluish staining. There was no need for us to regard the free Russell bodies encountered as anything but a displacement artefact, although the freeing of Russell bodies from plasma cells has been thought to occur *in vivo*.

Numerous "vacuoles" of varying size are often interspersed between the cells, frequently riddling the background of diffusely spread cytoplasm, (" . . . Semblable à un tapis troué . . ." Weil, et al., 1936) (*vide* fig. 120). When of large size *they may be confused with spaces occupied by red cells, which have subsequently haemolysed*. If the cells have been subjected to considerable trauma these "vacuoles" may appear superimposed on nuclei and even forced into them to varying depths (figs. 120, 121). Here again one is confronted with the difficulty of distinguishing artefacts from pre-existent structures. "Vacuoles" have been observed in the nuclei of lymphocytes: they have been described by Schrek (1947) who observed them as reaction to X-ray injury, and who supposed them to be foci of water absorption; Osgood and Ashworth (1937) speak of "fenestrated lymphocytes" in cases of infectious mononucleosis in man. The appearance shown in fig. 120 may also be interpreted as emergence of vacuoles from the nucleus. If these vacuoles contain granules, they may resemble the intranuclear blood platelets already described (cf. fig. 122 with plate I, figs. 14 and 21), although one is never sure that this appearance may not represent the final stage in platelet digestion. Where the vacuoles are very big, they may give rise to appearances reminiscent of some of the "L.E." cells of Hargraves, Richmond and Morton.

The vacuoles do not stain with Sudan IV, or with Sudan Black. Their frequent occurrence in large numbers makes it difficult to believe that they are *all* pre-existent structures liberated from cellular cytoplasm: it is more likely that some of them merely represent areas where the smeared out cytoplasm has not become confluent; those which lie over or partially in nuclei must possess substance.

B. *Contaminant Artefacts.*

1. *Endogenous Contaminants.*

The most frequent endogenous contaminations of routine smears include striated muscle fibres (sometimes containing sarcospores), kidney cells, liver cells, squamous epithelium (from the skin, sometimes presumably also from that of the person who made the smear). Such elements most usually arise from the use of a knife previously employed to incise other organs. The question of serosal cells has previously been dealt with. The ability to recognise some of these elements is of practical importance, as it is advisable to be aware during routine examination whether one may not be dealing with e.g. kidney, liver or lung smears—often erroneously labelled as spleen or gland smears. It seems to need pointing out that *to accept such smears uncritically and patiently to scrutinise epithelial cells for the presence of Koch's bodies is a mere waste of time.*

2. *Exogenous Contaminants* (Plate VII, figs. 123-127).

Exogenous contaminations often preponderate over and obscure the elements of the smear, as the routine worker knows to his cost. They comprise almost every conceivable type of "misplaced matter", but in diagnosis some assume more importance than others. Bacteria which are mere contaminants have to be distinguished from pathogenic organisms, and also preferably from putrefactive organisms, so that the two diagnoses "unsuitably prepared smear" and "decomposed" are not indiscriminately returned.

The resemblance of colonies of cocci (fig. 123) to basophil granules has already been commented upon. As has been mentioned, haemosiderin and haemofuscin should not be identified as "dirt". Bodies such as shown in fig. 124 [öospores of

certain fungi[29])] are not uncommon contaminants of smears, and deserve mention on account of their very characteristic and beautiful appearance when stained with Giemsa, the exine envelope being brilliantly basophilic and of luminous appearance. Amorphous dust-like particles originally of vegetable derivation are similarly basophilic and could be confused with haemofuscin. Fungus hyphae are readily recognisable. Some of the spores[29] (figs. 125, 126 and 127), when isolated, are not so easily recognised, and as mentioned they may simulate atypically stained erythrocytes (fig. 126). Others (fig. 127) bear some resemblance to certain protozoan parasites (e.g. Leishmania) and also to "Jolly body plasma cells" (cf. fig. 127 with plate II, fig. 49).

C. *Artefacts Arising Subsequent to Smear Preparation.*

1. *Stain Deposit* (Plate VII, figs. 128-130).

This is exceedingly common in routine smears and varies greatly in degree of fineness and depth of colour, i.e. depending on whether the neutral or the basic dye component is deposited. Some extremes are illustrated by figs. 128 and 129. When limited to the red cells, as sometimes occurs (fig. 130), stain deposit may be mistaken for punctate basophilia, or even for eosinophil or basophil granules.

2. *Grease Pencil Marks.*

Under high magnification blue grease pencil marks may greatly resemble haemofuscin, and may actually be confused with it, until the examiner becomes aware of the parallel streaks forming part of a mark.

3. *Scratches* (Plate VII, fig. 131).

Light scratches made over the nucleus of a stained cell *remove the adsorbed shell of azur-eosinates revealing a blue-tinted core* (fig. 131). [Appearances such as these led Von Prowazek, 1914, probably rightly, to consider Giemsa staining of nuclei, amongst other structures, as a double process: (*a*) mordanting or prestaining by crystalloid azur, and (*b*) precipitation of colloid azur-eosinate.]

D. *Miscellaneous.*

1. *Autolysis* (Plate VII, fig. 132).

It has been thought advisable to illustrate the characteristic appearances due to autolysis, especially as affecting nuclei of the lymphocyte series of cells (fig. 132). The accentuation and clumping of the basichromatin is well seen here. This phenomenon does not seem to be widely appreciated. It should be compared with necrobiosis, which, however, is not dealt with in this article on the appearances of the normal spleen.

2. *Unclassified* (Plate VII, fig. 133).

In all, the appearance shown in fig. 133 has been encountered three times in routinely prepared smears. It is revealed as a neat regular cluster of chromosome-like bodies, each constituent structure (actual chromosome?) resembling an indian

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[29] These identifications must be looked upon as coming from animal cytologists not versed in mycology. To Dr. M. G. Mes, of the Department of Botany, University of Pretoria, we are indebted for correcting our erroneous conception of the structure shown in fig. 124 as being a pollen grain, and to Dr. P. H. B. Talbot, of the Division of Botany of the Department of Agriculture. Pretoria, for his warning that we could possibly be incorrect in assuming the bodies shown in figs. 125 to 127 to be spores of fungi.

club, with centrally disposed head and a distally disposed, sharply squared end, as if the handle of the club had been cut short. We have come to no definite conclusion regarding its nature. It is inconceivable to us that it represents a mitotic metaphase of either any somatic cell or a contaminating cell whether of plant or animal nature. Dr. P. H. B. Talbot (see footnote 29) ventured the suggestion that it possibly may be a phialide of a mould. At most this structure is offered as a target for opinion.

Chapter IV—Discussion.

1. *Note on the Lymphocyte and the Monocyte—Macrophage Problem.*

(i) *The Phagocytic Activity of Lymphocytes.*

For the bovine species the " macrophage " par excellence *proves to be the lymphocyte in a phase of phagocytic activity. And these lymphocytes should not be confused with monocytes.*

In spite of surprisingly numerous previous observations (foremost amongst them being those of Maximow who taught at least that his " polyblasts "—macrophages of most authors—were of lymphocytic origin) any ascription of phagocytic powers to lymphocytes is unorthodox. Foot (1919) frankly discussed the possibility, but virtually rejected it in the words: " Do lymphocytes possess the power of phagocytosis? I think not ". Bunting (1938) in his review on the functions of the leucocytes clearly states that the lymphocyte, although amoeboid, is not phagocytic (p. 442).

This well-entrenched and widespread idea probably originated from Aschoff's exclusion of the lymphocyte from his " Reticulo-Endothelial System ". Bloom (1938a) very aptly remarks on this as follows: " . . . it is curious that this point of view is based largely on the work of Kiyono, who on page 95 of his 1914 monograph admits that some of the exudate cells come from hypertrophying emigrated lymphocytes ". And again: " Kiyono, in later work with Nakanoin (1919) brought further proof of the ability of the lymphocytes to hypertrophy ".

We may also cite Kiyono's (1914b) statement regarding " splenocytes ". " . . . diese Speichern . . . sehr stark die vitalen Farbstoffe." But for this author splenocytes are not lymphocytes (see below); in support he mentions that the lymphocytes of the Malpighian corpuscles are hardly ever (" so gut wie nie ") phagocytic. This observation is indeed a very common one, but none of the authors who have made the statement have ever paused to consider the peculiar vascular arrangements of the spleen, whereby only lymphocytes of the pulp are bathed in blood, those of the follicles being at most in contact with plasma filtered out from the follicular capillaries. This author further states: ". . . muss ich die Möglichkeit zugeben, dass ein Teil der lymphatischen Zellen sich zu Histiozyten umwandeln kann, vor allem bei Entzündungen, wenn auch der exakter Beweis schwer zu erbringen ist ". Bergel (1930) cites Kiyono (1914a): " . . . an anderen Stellen des Körpers, und insbesondere in der Blutbahn, Übergänge von Lymphozyten und Histiozyten vorkommen ".

In their earlier work Aschoff and Kiyono (1913) admitted that " Histiozyten bei entzündlichen Reizungen zu immer kleineren Lymphozyten ähnlichen Zellen sich umformen ", and therefore they have to distinguish between these small histiocytes which retain phagocytic potentialities (Karminspeicherung) and small lymphocytes. They bolster up this distinction by the statement that plasma cell derivation from these small histiocytes has not been proved.

Recently Rebuck (1947), in a review on the functions of the leucocytes, marshalled a most impressive array of champions against this aspect of the Aschoff-doctrine, beginning with Metchnikoff. He adds certain observations of his own (not published at the time) on the rapid hypertrophy of individual living lymphocytes of man into small macrophages in warm-stage preparations. To Rebuck's list one may add Ziegler (1910), Fazzari (1926), Bergel (1930) (in addition to his 1920 publication), Katzenstein (1931), Severeus, Roberts and Card (1944), and Pliske (1946), and in a way Alexeieff (1925b), and Erdmann, Eisner, and Laser (1926). [See also Klemperer, 1938, fig. 27 (*f*).]

Rightly Jaffé (1938) states: (p. 1026) " The resistance against this interpretation is more a matter of tradition and analogy to other blood cells than of actual observation ". So great is this resistance, that where phagocytic cells obviously related to lymphocytes have been observed, the doctrine has usually been watered down by merely saying that the phagocytes are of lymphocytic derivation. They then receive a new name: a polyblast or a macrophage may be phagocytic, not a lymphocyte. As instances hereof we may cite, apart from the well known works of Maximow (*vide* Maximow, 1927c), Hertzog, (1938), who distinguishes a pre-phagocytic stage in lymphocytes prior to their morphological differentiation towards a definite macrophage, and adds that pre-phagocytic stages may occasionally show phagocytic activity under experimental conditions. (This is in contrast to Strumia and Boerner (1937), who are emphatic that lymphocytes never show phagocytosis.) De Kock (1923) conservatively describes well-figured typical lymphocytes with phagocytosed red blood corpuscles as being " of the nature of lymphocytes ". Bloom (1938a) still indicates the direct transformation of lymphocyte to macrophage with a question mark. (His scheme 13, p. 434.)

McJunkin (1918) definitely excludes lymphocytes as macrophages by definition: " If it were not for the carbon within these cells, however, some of them would be mistaken for large lymphocytes, though most of them would fall in the classes commonly known as transitional and large mononuclears ". Note too, his annotation to fig. C of his appended plate: " The phagocytic cell resembles the lymphocyte morphologically ". One cannot escape the strong suspicion that some of his hyaline cells (McJunkin, 1925a) or of his " haemendotheliocytes " and " lymphendotheliocytes " (1925b) may be lymphocytes. More especially is this so if one considers the now fairly generally accepted non-specificity of neutral red granules and rosettes.

Sabin, Doan, Cunningham and Kindwall (1925) found a discrepancy in the lymphocyte-monocyte ratio between smears and supravital preparations of human blood. Doan and Sabin (1926) again remark on this, on finding a similar discrepancy in the case of the rabbit. They conclude that small and medium clasmatocytes had been overlooked as being such in supravital films, and had been classified as lymphocytes in fixed films. To state the case from our point of view, these authors regard cells morphologically similar to lymphocytes as clasmatocytes, and that on account of their neutral red reaction. It is noteworthy that all their " clasmatocytes " that were filled with red cell fragments were found to be negative to the peroxidase reaction, both in rabbits and in humans. In the spleen of the rabbit Sabin, Doan and Cunningham (1925) also describe a "small type of clasmatocyte " distinct from the monocyte. The reader is also referred to Wehrle's (1938) and Bloom's (1928a) descriptions of the type of cell concerned in erythrophagocytosis (*vide pages* 121 *and* 122).

We shall conclude these citations from the literature by quoting Pappenheim (1913), namely: " . . . dass es viele Formen und Arten von Makrophagen gibt

und dass der makrophagen Charakter nur ein morphologisch bestimmter Funktionszustand, ein durch funktionelle Anpassung erworbener Zustand verscheidener einander genetisch naheverwandten, aber nicht ohne weiteres identischen, lymphoiden Zellformen ist ".

We believe we have shown that a most important of the hitherto very obscure functions of the lymphocyte is that of phagocytosis of other blood elements.[30] *This function is displayed in the environment of the spleen—perhaps elsewhere.* Maximow (1909, 1924, 1927c) takes up the viewpoint that the lymphocytes must pass a period of maturation in the circulation before being capable of developing into polyblasts. His pupil Tschaschin (1914) associates himself with this idea because of his inability to demonstrate vital staining of lymphocytes of spleen and lymph nodes. He, therefore, postulates a state of immaturity for these cells, as does Downey (1916-17), who also found lymphocytes of the lymph nodes not taking up vital dyes. It is impossible at this juncture to make a hard and fast statement in how far hoarding of vital dyes is an index to phagocytosis of body elements.

The fundamental concept of " unripe " cells being turned out from the blood-forming organs to manifest their functions in the circulation seems thus to demand attention, if not, revision. So far as the lymphocyte is concerned this cell displays little evidence of function in the bloodstream; but only when subjected to special conditions (ligation of vessels, excessive demand on those organs which are concerned in the immunological response in addition to haemopoiesis) or when it emigrates into inflamed tissue, or is in a tissue environment with specific circulatory relations, does it exercise its potentialities.

We have observed erythrophagocytosis by lymphocytes in the peripheral circulation under pathological conditions (protozoal infections) and then only infrequently. This may be due to " spilling " from the spleen of phagocytosing lymphocytes which have already carried out their function in an appropriate habitat. Erythrophagocytosis in the peripheral circulation has been observed by Mallory (1898), Miller and Pepper (1916), Huck (1923), Weill (1923) and by Taliaferro and Klüver (1940) amongst others, in the course of an infectious disease (typhoid, malaria); in haematological disorders (sickle cell anaemia); or after experimental procedures (injection of typhoid bacilli).

It has also been noted after splenectomy, usually during some post-splenectomy reaction to an infection (Domagk, 1924, Lepehne, 1918, de Kock, 1923, Hirschfeld and Sumi, 1925, Lauda, 1925, Paschkis, 1926). Usually " monocytes ", " large mononuclears " or " endothelials " are the supposed phagocytes. Some of them, where illustrated, do look like lymphocytes or moblised reticulum cells. Taliaferro and Klüver are the only ones to mention phagocytosing lymphocytes, as has been referred to before.

On Doflein and Reichenow's (1929) unusual interpretation of " Abtötung ohne Einverleibung " of trypanosomes by lymphocytes in the cerebro-spinal fluid in cases of sleeping sickness, we are unable to offer comment.

Bearing the specialized type of phagocytic function of the lymphocyte in mind (as well as its antibody producing potentialities—*vide* Dougherty, Chase and White 1944, Harris, Grimm, Mertens and Ehrich 1945, Kass 1945) *the whole question of lymphocytic function might well be re-investigated: the lymphocyte in the reaction zone of inflammation, as an infiltrating cell of neoplasms, in its association with and migration through mucous membranes.* (*Vide* Bunting and Huston, 1921; Andrew and Andrew, 1945; Andrew, 1946a; Andrew and Collings, 1946).

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(30) We avoid the time-honoured qualification " worn-out " blood elements, because it seems to be largely habitual and not based on exact knowledge, lacking morphological criteria. Its use may be quite correct, but is at present purely inferential.

(ii) *The supposed lymphocytic derivation of the monocyte.*

Our findings and experience with bovine spleen smears lend no support whatever to the possibility that the conclusions of Bloom (1927-8, 1928a, 1928b) might apply to the bovine species. His studies on the origin of the monocyte were made on rabbits suffering from monocytosis evoked by *L. monocytogenes* or by injection of vital dyes, etc. He concluded that the monocytes arise by direct transformation of lymphocytes, a standpoint championed by Maximow in his classical works extending over a quarter of a century. It might be expected that, if Bloom's conclusions in respect of the rabbit were universally valid (which indeed he himself seems strongly to imply) then they might well be expected to hold for the bovine, a species in which it is wellknown that it is not easy sharply to distinguish some of the blood monocytes from lymphocytes. But in the spleen smear of the ox one sees unmistakably that the immediate precursors of monocytes are " undifferentiated " free reticulum cells. Bloom (1928a) concludes that " monoblasts do not exist ". While we are not enthusiastic about the desirability of labelling any cell a monoblast, the term may be not unsuitably applied to the intermediate stages between reticulum cells and monocytes. We for long accepted, and one of us always taught, since Bloom's work, that at least one origin of the monocyte was from the lymphocyte. But our studies of the bovine spleen and unpublished observations on cases of reticulum cell sarcomatosis of the dog (which might quite fittingly be termed monoblastic aleucaemia) led us to abandon this view for the domesticated animals, and to return to a view more in line with that of the majority of human haematologists[31] (of whom Bloom is critical) and since Bloom's work again supported by Karmally (1929), Forkner (1930), Seemann (1930), (1931), Cattaneo (1931), Ehrich (1934) and Doan and Wiseman (1934).

Prior to this Lang (1926b) had stressed the role of the undifferentiated mesenchymal elements as stem-cells in extramedullary myelopoiesis affecting the spleen and lymph nodes. Besides the lymphocyte origin, Maximow (1926, 1927c), and Lang (1926a, 1928) admit the development of the monocyte from the undifferentiated mesenchymal cell. Bloom (1938a) only admits this as a theoretical possibility and strongly criticises Forkner's and Ehrich's work. The whole matter is still in a state of flux. Latta and Johnson (1934) have championed the strictly unitarian viewpoint, supporting Maximow (1922) as to the stimulation to cell differentiation afforded by splenic extract when added to cultures of lymphatic tissue, in contrast to Ehrich's (1934) and Yamaguchi's (1931) negative results in this respect.

Conway (1937) again supports Bloom, whereas Bueno (1947) indicates the origin of monocytes from undifferentiated cells at the marginal zone of the Malpighian follicles of the spleen of guinea-pigs suffering from Brucellosis.

Herbut and Miller (1947) in their study on the histopathology of monocytic leucaemia state: " R.E. cells are unquestionably the precursors of monocytes ". In the Schilling type of monocytic reticulo-endotheliosis this origin is usually obvious (see Downey, 1938).

Those who deny the lymphocytic derivation of monocytes, also directly or indirectly deny the phagocytic potentialities of the lymphocyte. In this respect we differ. Furthermore we adopt a different viewpoint from the Aschoff school as regards origin of monocytes from " histiocytes " (i.e. in the case of the spleen from activated reticulum cells). Our differing concept of what should constitute the R.E. system will be set out in sub-chapter 5 below. With the basic idea expressed

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(31) *Vide* Cunningham, Sabin and Doan (1925), and Masugi (1927).

by Muir (1902) we agree: "Although these different forms of nongranular cells behave in a somewhat similar fashion so far as their phagocytic properties are concerned, and may become altered in appearance as to be indistinguishable from one another, it does not follow that they can be transformed the one into the other".

(iii) *The status of Monocytes in the Spleen.*

Due mainly, we believe, to rather narrow preconceived ideas, the identification of the predominant type of nucleated cell in the splenic pulp has in the past given rise to much difficulty, especially during the first three decades of this century. (Yet Helly in 1906 had already stated that the splenic macrophages are partly of lymphocytic derivation, and partly originate from the reticulum cells.) The now discounted concept of "splenocytes" as a specific and separate cell type, postulated by Türk (1904, 1912) amongst others, and propagated by prominent haematologists of that time (e.g. Schridde, 1909), is an example of this. On account of the fact that the pulp cells take up lithium carmine in the vitally stained animal, Kiyono (1914b) was forced to classify them as "Gewebshistiozyten, welche ihrer Form und Gestalt nach den grossen Mononukleären und Übergangsformen gehören", and he spoke of them as "kleine und mittelgrosse Histiozyten" with marked resemblance to "vital ungekörnte grosse Mononukleären", so much so that ". . . . die Möglichkeit nicht aus (zu) schliessen (ist), dass auf dem Wege über diese Formen ein Übergang von den Lymphozyten zu den Histiozyten im zirkulierenden Blute stattfindet"—albeit in limited measure. Thus it is manifest that one runs into great difficulties, when wishing to distinguish between lymphocyte, monocyte and histiocyte by applying for this purpose narrowly predefined interpretations of vital staining.

Tschaschin (1914) regards the "splenocyte" as a functional variant ("Abart") of lymphocyte. Although he could not demonstrate any vital staining of splenic lymphocytes by trypan blue, isamin blue or collargol, he stressed the close relationship between, if not actual identity of, lymphocytes and histiocytes by virtue of the former's ability to transform into the latter.

Paremusoff (1911), in Giemsa-stained smear preparations and sections mainly from guinea-pigs, found the pulp cells not characteristic of real monocytes, but of myeloblastic lymphoidocytes ["myeloisch granulopotente lymphoidozytäre und leukoblastische Zellformen" (!)]. His very good illustrations show myeloblast-like elements. Probably this haemocytoblastic cell type is as species-specific an appearance as Downey (1927) found for the lymph nodes of this animal. It may be added here that the phagocytes Paremusoff found were rare. His description is confused. (They are supposed to resemble large mononuclears, but are nearer endothelials, with nuclei resembling myelocyte-like leucoblasts). He derives them from the "Serosa-endothelien" without offering much by way of proof. Yet his figure 48 (plate 9) looks like a lymphocyte, whereas his figures 46 and 47 resemble activated reticulum cells.

Pappenheim (1913) and Pappenheim and Fukushi (1913) postulated that morphologically specific splenocytes do not exist, but that these cells are functionally specific "myelopotent macrolymphocytic cells" morphologically similar to leucoblasts and "macrolymphocytes". Pappenheim (1913), led by Paremusoff's results, apparently unable to look upon the lymphocyte as being phagocytic, and misled by his inability to demonstrate "Speicherung" by cells of the circulating blood (see Downey, 1916-17, 1917, for an explanation) erected a complicated schema, whereby the splenic pulp was to consist predominantly

of histiocytes, of monocytes derived from them, and of myelopotent macrolymphocytes formed in turn from the latter, in addition to " leucoblasts ". The monocytes, functioning only as parent cells for these myelopotent macrolymphocytes, and the latter functioning as special " haemolysin-formers ", supposedly remained sessile in the spleen. The blood monocytes were not with any ease of mind to be derived from the spleen.

Pappenheim and Fukushi (1913) conclude, after making comparative leucocyte counts on the blood from the splenic artery and vein, that all adult types of monocytes in the spleen are accounted for by a normal filtering out process from the blood. We are quite prepared to accept that such filtering or immigration may occur.

McJunkin (who denied any ability of the lymphocyte to take up neutral red) found in the spleen of vitally stained rabbits subsequently injected with Indian ink (1925a) that mononuclear cells with typical rosettes were infrequent, certainly never present in large numbers. Younger forms of the rosette type of cell were not seen: neither in the pulp, nor at the periphery of the lymphoid follicles. However, types with scattered neutral red granulation were fairly numerous in smears and especially in sections, both within and between well-defined sinuses. (Coming from a staunch supporter of the endothelial origin of phagocytes, the latter statement is important). These cells appeared free, or semi-detached. Some contained carbon particles, and of these some showed an irregular neutral red granulation, others no granulation at all. We believe that the majority of these cells were lymphocytes (see page 113), and that monocytes were included among the true rosette forms only.

Sabin, Doan and Cunningham (1925) found between 7 per cent. and 50 per cent. of monocytes with an average of 18 per cent. in the rabbit spleen.[32] Weil et al. (1936) gave a figure of 5—10 per cent. for the normal human splenogram, although their illustrations depict rather a type of lymphocyte than monocytes, and do not at all correspond with their description.

In contrast, Erdmann, Eisner and Laser (1926) found that the rat spleen contains but few monocytes. Splenocytes do not constitute a specifically defined cell group, but include lymphocytes, monocytes in very moderate numbers, and plasma cells. Hu and Ch'in (1932-33) could find no monocytes in the normal rat spleen (supravital studies). Masugi (1927) and Forkner (1929, 1930) failed to find significant production of these cells in the rabbit spleen. Tempka and Kubiczek (1938) found only 0·5 per cent. of monocytes in human spleen smears. Naegeli (1928) did not see " grosse Monozytenhaufen ".

Apart from the findings of Weil et al. (loc. cit.) and Sabin, Doan and Cunningham (loc. cit.) we have not found in the recent literature any indications contrary to the views of the authors quoted in the preceding paragraph. The bovine spleen is thus not unique in its paucity of monocytes.

2. *Note on Thrombophagocytosis.*

This process, as far as we have been able to ascertain, was first definitely described by Bernhardt in 1913. The difficulty of observing it in sections was stressed by him. Sublimate-alcohol fixed, and paraffin-embedded material is

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([32]) The latter authors have described the stages of monocyte formation from the primitive stem cell in this species (Cunningham, Sabin and Doan, 1925). For a discussion of this aspect see page 113 where the work of Doan and Sabin (1926) is dealt with, as well as Hall's (1938) evaluation of the supravital method.

apparently a prerequisite. It was observed mostly in cases of scarlet fever, but also in typhus and in cholera nostras. Platelets were found lying free in sinuses and in pulpa of the spleen, but not with any certainty in the Malpighian follicles. (This is to be expected.) The intracellular platelets were in large, usually round cells, with one, often more, round or vesicular nuclei, frequently together with red cells, and pigment. The platelets were also observed in "resting" (i.e. undetached) reticulum cells, although not often. In the sinus-cells they never occurred in healthy apes, but only in acute splenic tumour (no illustrations given). Platelets were also found in Kupffer cells of the liver and in "den Pulpazellen gleichenden Monocyten der Mesenteriallymphdrüsen". Bernhardt's figures 2 and 4 are to us mobilised reticulum cells, as his description suggests. His fig. 5 is definitely a lymphocyte, his fig. 3 probably a hypertrophied lymphocyte ("polyblast").

Since Bernhardt, the only definite descriptions we can find regarding thrombophagocytosis in the spleen are those of Cori (1922), Seeliger (1924), Domagk (1924), Alrutz, Nortell and Piette (1926), and of Tempka and Kubiczek (1938). Both Cori and Seeliger are quoted by McKay (1931) as having observed the presence of phagocytosed and degenerated platelets within the "endothelial cells" of the spleen. Alrutz, Nortell and Piette described granules staining blue with methylene azur but practically invisible with haematoxylin eosin in the reticulum cells and endothelial cells of the spleen in a case of thrombocytopenic purpura. These they interpreted as degenerated platelets. Their single illustration is not at all clear. In the case of the endothelial cells we strongly suspect the observed granules to be "haemofuscin". They claimed also to have seen better preserved platelets in the cytoplasm of the endothelial cells (not illustrated). Domagk (1924) found "ein auffälliger Reichtum an Bluttplättchen" in the "endothelial cells" of the rat spleen, whilst Tempka and Kubiczek (1938) stated that thrombophagocytosis is minimal in man and is carried out by reticulum cells. In the rabbit, however, they found thrombophagocytosis to be more pronounced: illustrations are given. According to them thrombocytolysis is the essential and more or less invariable mode of platelet destruction. This they also illustrate, but again poorly. Apart from this, thrombocytolysis has to our knowledge only been reported upon by Kaznelson (1916), who described it as being accomplished by the R.E. cells of the splenic sinuses in cases of essential thrombocytopenic purpura. As we had no direct access to this work, "thrombocytolysis accomplished by R.E. cells", in contradistinction to thrombophagocytosis, remains to us a rather mysterious process. In a later publication (1919) he is quoted by MacKay (1931) as having seen platelet phagocytosis in the spleen in one case of pernicious anaemia.

For the rest, research workers are very diffident about their findings on supposed thrombophagocytosis, and this is quite to be understood. As Hertzog (1947) put it: "others have failed to find this phenomenon. It is extremely difficult to study phagocytosis of platelets in paraffin sections". Hertzog and Roscher (1921) were not quite sure of fragments in macrophages of the spleen and liver, but in general they were against accepting thrombophagocytosis as occurring. Forkner (1929) found cells "resembling clasmatocytes" containing bodies "resembling platelets" in the mesenteric lymph node of the rabbit. Pardi (1915) was not satisfied that the presence of material resembling platelets within phagocytosing cells was good evidence of phagocytosis.

Hirschfeld and Sumi (1925) described thrombophagocytosis in the peripheral blood of rats. The phagocytes were mostly "endothelials" (phagocytosing lymphocytes and mobilised reticulum cells according to our interpretation of their illustrations.)

Beyond these findings, statements on the relation of thrombocytes to the R.E. system have been limited to speculation and inference. Bedson (1926) concluded from the high platelet counts he observed after splenectomy and R.E. blockade, that platelet phagocytosis (by R.E. cells) in the spleen was a prominent function of this organ. No morphological studies were undertaken by him. Koster (1926) also found in guinea-pigs a sharp rise in blood platelet count after R.E. blockade by trypan blue. This was followed by a drop, despite further dye injections. He concluded that phagocytosis by R.E. cells is not the only method of disposal of platelets.

At this juncture we wish to draw attention to the fact that, notwithstanding the paucity of literature dealing with this aspect, or perhaps just because of it, some authors have illustrated thrombophagocytosis without describing, or *aliter*, without being aware of it.

In this respect the reader is referred to Jochmann and Blühdorn's (1911) plate 7 purporting to illustrate inclusions regarded as very characteristic of myeloid leucaemia, but, amongst others, showing cells looking more like lymphocytes containing platelets. (These cells were reported to be oxydase-negative and to contain no proteolytic enzymes). A platelet in an apparently intranuclear situation was also shown. Similarly, in the work of Taliaferro and Klüver (1940) may be seen a platelet phagocytosed by a macrophage (their plate No. 1, fig. 28).

For the sake of completeness mention must be made of the views of Holloway and Blackford (1924) and Bock (1932), who concluded that blood platelet destruction is no function of the normal spleen. Bock found, on the contrary, that passage through the spleen enhances the resistance of platelets, and that they were more numerous in splenic vein blood than in blood from the bone marrow. Deficiencies in technique may be responsible for this finding, but we do not feel competent to pass critical judgment. Spadolini's (1928-29) morphological observations of platelet formation in the spleen we can discount as being based on mis-appraisal of artefacts. (See page 84). His fig. 7 (1929) actually demonstrates the presence of (phagocytosed, not formed) platelets both in the cytoplasm and in the nucleus. Watzka's (1937) findings regarding platelet origin from sinus endothelium and reticulum cells we could not confirm; the possible origin from sheath cells should be considered. [See page 99, subheading (3), and page 124].

The demonstration of platelet phagocytosis in the spleen would afford a highly satisfactory confirmation of the arbitrary suspicion that—in some way or another not specified—platelets might be " destroyed " in the spleen (*vide* also Nickerson and Sunderland, 1937, who speak of " thrombocytolysis " in cases of thrombocytopenic purpura). That is, if the results of our findings on the bovine spleen could *mutatis mutandis*, be applied to man. It would also supply a rational basis for the empirical treatment of essential thrombocytopenic purpura by splenectomy. In view of Tempka and Kubiczek's findings mentioned previously, caution must be exercised. The supposed depressing action of the spleen on platelet formation (see Paul, 1942) must also be considered; we cannot discuss it here.

The fact that lymphocytes can phagocytose such a variety of elements makes it seem advisable to re-examine the origin of such phenomena as azur granulation. Although we have no particular suspicion that azur granules are the remains of the chromomere material of phagocytosed platelets, it would be a possibility—not yet taken into account in the existing literature on the subject—which demands exclusion. The Kurloff body problem likewise calls for re-examination, in the light of our widening knowledge of lymphocyte function.

3. *Note on Erythrophagocytosis.*

In the bovine spleen the scale of erythrophagocytosis and the fact that it is accomplished by lymphocytes are surprising.

Klemperer (1938, p. 1640-41) in a brief review mentions Kölliker's (1847) and Ecker's (1847) simultaneous discovery of erythrophagocytosis by "mononuclears" in the spleen as well as the subsequent opposition raised and the re-affirmation by Ponfick (1869) and by Kusnezoff (1873). He concludes that, while erythrophagocytosis is to-day generally accepted, the number of such cells found in the normal human spleen is very small.

Jaffé (1927) in his review of the R.E. system says: "In health phagocytosis of red cells is insignificant. I have never found erythrocytes enclosed in R.E. cells of normal human tissues". Of course, considering the classical view of what comprises the system of phagocytosing cells in the body, apart from possible species differences, this is scarcely to be wondered at. In any case the section method is most unfavourable for the recognition of erythrophagocytosis, let alone thrombophagocytosis.

Paton and Goodall in 1901 sketched the role of the spleen as scavenger of the blood. In the rabbit's spleen, Addison (1920) described iron-containing, "free-swimming splenocytes" which, under normal conditions, may occasionally show "what appears to be an entire corpuscle still intact within the splenocyte, but final judgment is often difficult". Ingested haemoglobiniferous fragments and leucocytes are also described. Bloom (1928a) (*vide infra*) was similarly not quite sure of the nature of "yellow vacuoles" found in the rabbit spleen during the course of *L. monocytogenes* infection.

In rabbits Steudemann (1914) saw but little erythrophagocytosis in the spleen. It was considerable only after long-continued, artificially produced, venous stasis. McJunkin (1925a) observed red cells and leucocytes in carbon- and neutral red-containing phagocytes in vitally stained rabbit's spleens. Andrew (1946b) found no erythrophagocytosis in the spleens of rats and only rarely in those of man. He concluded that this is apparently not a normal mechanism of red cell destruction. However, Domagk (1924) demonstrated erythrophagocytosis and leucophagocytosis in the spleen of the rat. Spadolini (1928) observed large mononuclears filled with red cells in the splenic vein of the dog.

Knisely (1936b) regarded erythrophagocytosis as merely an agonal phenomenon, but was strongly contradicted by MacKenzie and co-workers (1941), who gave a good description of this process. Tempka and Kubiczek (1938) stated that erythrophagocytosis in man is so rare as to be of no practical importance, whereas in the rabbit it is very pronounced. McNee (1931) stated (p. 954), "venous sinuses contain blood-cells of every variety, but in many healthy animals, and in various pathological conditions in man, large phagocytic cells containing red corpuscles or their debris are also found".

Doubtless there are order and species differences: the liver plays the most important role in red cell destruction in amphibia, birds and lower mammals, whereas splenic haemophages are of more importance in rodents and higher mammals, (Kyes 1914). According to Rous and Robertson (1917) phagocytosis of entire red cells in the spleen is most marked in guinea-pig, dog, and rat, variable in the rabbit, slight in man and monkey, and practically absent in the cat.

More definite and pronounced erythrophagocytosis occurs under abnormal conditions. Addison (1920) had no difficulty in demonstrating phagocytosis of injected washed pigeon blood-corpuscles in the rabbit's spleen; polymorphonuclear

leucocytes and myelocytes emanating from the bone marrow reacting to the injection of the foreign blood were similarly phagocytosed. Cary (1915) also demonstrated phagocytosis of bovine corpuscles in the rabbit's spleen. Wehrle (1938) gave a fairly extensive description of this fate befalling injected red cells and granulocytes. According to Muir (1902) and Klemperer (1938) erythrophagocytosis may be enormously increased in various diseased conditions.

Of course this method of removal of red cells is by no means the only one. Lepehne (1918) who examined this question extensively, had to admit possible extracellular breakdown of haemoglobin in rabbits. Muir (1902), and Epstein (1924), also indicated that erythrocytes may degenerate and lyse without previous phagocytosis. This humoral destruction of erythrocytes is stressed by Tempka and Kubiczek (1938). To Bock (1932) it is the chief method. As evidence, the preponderance of siderophages as compared to erythrophages is cited, as well as the presence of large numbers of " schizocytes " and " ghost cells " in the spleen. As to the first point, we agree that haemosiderin-containing cells are very numerous, also in the bovine spleen; but in the absence of reliable comparative counts, and until such time that one can prove that the haemosiderin is not derived from ingested red cells, we are forced to leave the question open. We feel that neither time factors nor technical shortcomings have been sufficiently considered in assessing the significance of the disparity between siderophages and erythrophages.

Fragmentation of red cells, first observed by Rous and Robertson (1917), has been observed *in vivo* by Doan and Sabin (1926). The former two authors also recorded the concentration of schizocytes in the spleen. The red cell fragments are then phagocytosed. This method, in contradistinction to the laking process, is supposed by them to be the chief method of red cell destruction.

Regarding the cell type which is responsible for phagocytosis, the reports are on the whole vague, the terminology varied and the illustrations, when given, often useless for cell identification. Briefly, the opinions may be summarized by stating that two types, a reticular and a mononuclear type, are held responsible. Addison (loc. cit.) described in the normal rabbit " free-swimming mononuclear splenocytes ", with round or oval, generally pale-staining nuclei, usually eccentric, with well-defined nuclear membrane and with chromatin in a network of fine lines with one or two large dots. Where haemolysis occurs due to injection of foreign blood corpuscles, very fine iron-containing yellow granules are found in the endothelium of blood channels, the iron-content always being less than that of the " splenocytes ".

Doan and Sabin (1926) mention the clasmatocyte as the chief cell, both large and *small* " clasmatocytes " being involved in the process (Sabin, Doan and Cunningham, 1925.) Wehrle (1938) states that a large proportion of the phagocytes are " mononuclear cells of small size situated in the cords of the pulp " which " often contain single engulfed erythrocytes . . . The phagocyte is often so small that its cytoplasm forms a narrow and inconspicuous zone about the nucleus and the ingested corpuscle. The nucleus of this cell stains more deeply and homogeneously than that of the larger phagocyte. The engulfed cell is pressed against the nucleus and often lies partially within an indentation of the nuclear membrane ". This cell is casually referred to as a " monocyte ". The large phagocytes are described as being of varying shape, oval, polygonal, elongate or stellate, with processes in intimate contact with reticulum fibres, and containing a large, vesicular pale nucleus differing conspicuously from the previous cell type. Comment is unnecessary.

Bloom's (1928a) illustration of certain appearances in moist-fixed spleen smears from rabbits infected with *L. monocytogenes* leaves no doubt that he was dealing with erythrophagocytosis by lymphocytes. He does not, however, go further than to say: " One of the very striking findings is the presence of phagocytosed material—usually recognisable as probably red cell dèbris— in cells which are typical lymphocytes in other respects . . . Similar yellow vacuoles—possibly ingested red cells—may be found in cells of the small and medium lymphocyte classes. They may also occur in cells which are obviously but slightly changed large lymphocytes ". This pronouncement of a distinguished haematologist speaks for itself.

Leucophagocytosis we have very occasionally observed. It has been described by Werzberg (1911), Paremusoff (1911), Steudemann (1914), Lepehne (1918), and by Sjövall (1936), besides the authors incidentally mentioned previously. The reticular histiocyte has been held responsible, whereas we have seen it in a phagocytosing lymphocyte.

4. *Note on the Schweigger-Seidel Sheath Cells.*

In the literature an appreciable number of authors have in a more vague or more definite way indicated the nature of the sheath cells as nothing but condensed pulpa reticulum. The older authors (Carlier, 1895, Greschik, 1915, Neubert, 1922, and Oberniedermayr, 1926) seemed more impressed by the condensations of reticulin in the sheaths. The sheath cells, however, were the object of many a guess and a name (e.g. Neubert: " sesshaft geworden weisse Blutkörperchen.") Even Riedel (1932), in an excellent description of the sheaths of the cat's spleen, dared not commit himself further than " Rundzellen ". To these authors' names may be added those of Ono (1939) (mesenchymal condensations in the developing spleen of the embryo); Staemmler (1925) (growth centres for the pulp); Hueck (1930) (intimate connection with pulp, myelopoietic potentialities); and Jäger (1929) (dense reticulum).

Already in 1929 Li, Garven, and Mole, without having any qualms over the matter, had described the sheath of the dog's spleen as composed of R.E. cells, usually containing pigment. More pointed descriptions and thus more definite statements in this respect have come from Watzka (1937), Solnitzky (1937), and Bargmann (1941).[33] They look upon the sheath cells as typical reticulum cells, more closely bound towards the centre, and showing a more open or reticulated juxta-position towards the periphery, gradually merging into the reticulum of the pulpa. This change in configuration from centre outwards is also commented upon by Riedel (loc. cit.) and Loreti (1935). The close intimacy between these cells and the dense network of reticulin fibres is generally agreed upon.

The cytology of the sheath cells has received scant attention. The protagonists of the reticulum cell nature of these elements are emphatic that there is no difference (Solnitzky) or imply that it is very insignificant. Watzka speaks of an epithelioid appearance widely different from that of reticulum cells only in the case of the mackerel. Generally a vesicular nucleus is described, poor in chromatin, (which is finely granular and dispersed) and lying in a kind of syncytial cytoplasm or embedded in a very granular ground substance (Watzka, Riedel, loc. cit). The close continuity is disjointed and the cells are more

[33] The work of Loreti, F. and Sabbia, L. (1942) on " Structure and function of capillary sheaths of the human spleen ", *Haematologica,* Vol. 24, pp. 371-402, was not available to us.

discrete and dispersed with increase of blood content of the spleen (Hueck, 1930, Watzka, loc. cit.), or as result of experimentally produced splenic distension (Riedel, loc. cit.).

Solnitzky (loc. cit.) ascribes the syncytial appearance to bad fixation only. In smears we find that the syncytial arrangement is very pronounced (fig. 65); yet in perfused, Zenker-formol fixed preparations, definite cell membranes can be made out, and narrow intercellular canaliculi containing an occasional erythrocyte may be seen. We are of opinion that both good fixation and a certain amount of distension are required to reveal these details.

Regarding similarity between sheath and reticulum cells we believe that most of the descriptions stressing this point may be reconciled with our findings on the following grounds:—

(1) The sheath cells are morphologically very labile, reacting rapidly to physical alterations of splenic size and circulation—Mackenzie *et al.* (1941) liken the sheaths to rubber sponges. One would thus expect quite a difference between cells in smears obtained from post-mortally contracted spleens and those seen in preparations previously perfused *in situ*.

(2) Smears allow of a more critical discrimination between closely related cell types.

That differences are observable between the two types in the higher mammals may be inferred from (1) the fact that in the older literature the nature of the sheath cell was subject to controversy whilst the reticulum cell was a definitely recognized entity;[34] (2) the differences existing in lower animals (*vide* Watzka, and Bargmann, loc. cit.); (3) illustrations given, even by those who strongly champion the morphological identity of reticulum cell and sheath cell, which clearly show differences in appearance. In this respect the reader is referred to Solnitzky's figure 6 (plate 3) and Bargmann's figure 12 page 645. Riedel actually figures two types of sheath cells (his fig. 10). The more typical sheath cell with darkly staining nucleus he labels reticulum cell, the more vesicular type, "round cell" (!) The sheath cells which this author features as being in a more dispersed phase due to experimental distension of the spleen (his figs. 12 and 14) correspond well with our plate III, figures 66 and 67. Imai (1940) whose work was not available to us, definitely distinguishes two cell types, a more central and a more peripheral one, a distinction Bargmann considers unnecessary.

Regarding function, the numerous and diverse views are discussed by Solnitzky (loc. cit.). Phagocytosis is expected to be one of the functions or potential functions of the sheath cells by very reason of their nature. The process of mobilisation (which we have tried to sketch by the expedient of distinguishing two cell types morphologically) is an expression of evolution of phagocytic function and its normal consequence, namely desquamation. Phagocytic activity has been ascribed to the sheath cell by numerous authors (e.g. Staemmler, 1925, Li, Garven and Mole, 1929, Mills, 1926, Solnitzky, 1937, Bargmann, 1941).

Tait and Cashin (1925), who were more impressed by the valve-like action of the ellipsoids, non-committally indicated a mechanical choking of the sheaths by india ink particles, after this had been added to the perfusate; what we have

[34] Weidenreich (cit. Thiel and Downey, 1921) argued that the sheath tissues do not resemble reticulum in appearance and that the nuclei are much more irregular than those of reticulum cells.

no doubt is mobilisation of sheath cells, they looked upon as clearing of the choked pores of the ellipsoids by pulp phagocytes. Mills (1926), working in the same laboratory, at Tait's suggestion re-examined the role of the sheaths and showed not only their great phagocytic activity but also their mobilisation and subsequent hyperplastic regeneration.

Genuine erythrophagocytosis by non-mobilised sheath cells as opposed to mere passage of erythrocytes through intercellular canaliculi seems to be confined to species lower than mammalia (*vide* Bargmann, 1941, and Dustin, 1938); at any rate it does not, so far as our observations go, occur in the bovine.

Phagocytosis of bacteria has been mentioned by Ozaki (1917), who observed *S. aureus* injected into the perfused living spleen to be taken up by the outermost layer of the sheathed arteries.

The role of the sheaths as sphincters or valves in controlling the blood flow, which view has had so many champions in the past (e.g. Jolly, 1911, Loreti, 1935, Knisely, 1936a and b, to mention but a few), could not be investigated by us. MacNeal (1929) has pointed out the weak points in the evidence. The opinion of MacKenzie and co-workers (1941) that the sheath, in addition to their highly phagocytic potentialities, act as passive sponge-like regulators of blood circulation, seems to be a very sound one.

As mentioned previously, the resemblance of the rather rare larger granules seen in sheath cells to platelet chromomeres inclines us to attach some significance to Watzka's view that these cells are a source of platelet-like cytoplasmic fragments. If this view is correct, as well as his further deduction that these fragments, upon disintegration, may contribute to the plasma proteins (notably the globulins), the sheath cell would have to take its place in company with reticulum cells, lymphocytes, and plasma cells—elements which by various recent authors have been regarded as sources of antibodies. (See also our remarks on page 85, regarding the budding off of globules from mobilized reticulum cells, from which the sheath cells were indistinguishable, in a case of severe multiple protozoal infection).

5. *Note on the Reticulo-Endothelial System.*

As already indicated, we have come to find great difficulties in the use of this term. In the first place, the R.E. concept excludes lymphocytes, which we believe to be the most active of the phagocytosing elements in the spleen, at least under normal conditions. It likewise excludes such cells as neutrophils, in virtue of whose phagocytic powers our work has shown a similar theoretical, although minor, objection.

Secondly, to our surprise when we investigated the problem at first hand, we find no "reticulo-endothelial cell" in the traditional sense of the term, at least not so far as the spleen is concerned. Instead, we observe (*a*) reticulum cells and (*b*) sinus-lining cells, whose morphology and potencies seem quite distinct. The former give rise to lymphocytes, monocytes and free histiocytes (and presumably elsewhere, to any of the blood cells); the latter, in our spleen material, give evidence of *no further potencies whatsoever.*

We have with ease recognized circulating desquamated sinus cells in the blood of the ox under abnormal conditions in which monocytosis existed. The fact that they were recognisable certainly means at the very least that a monocyte is not a mere desquamated sinus cell; neither is a macrophage. (Cf. similar remarks made by Pappenheim, 1913, footnote pp. 180-1.)

We strongly suspect that the concept of the formation of macrophages and/or monocytes as desquamated sinus cells of the spleen may have been based on one of three types of erroneous interpretation. (*a*) The sinus wall has not been identified with absolute certainty as being sinus wall and not reticulum of the pulp. (*b*) Migration through the sinus wall by cells from the pulp of the spleen is wrongly interpreted as being *desquamation from* the sinus wall. To us indeed it seems to be only too easy to fall into this error, unless one has the alternative prominently in mind. (*c*) Cells which have become temporarily affixed to sinus walls (" lodged-out " cells in the sense of Eliot, 1926) might again loosen up and " desquamate ".

To enlarge on the first point: one cannot escape the conclusion, from perusal of the voluminous literature on the subject, that the venous sinus has been differently interpreted by different authors. There is a singular lack of precise correlation between findings of histologists interested primarily in the circulation route in the spleen on the one hand, and of haematological cytologists on the other. Tschaschin (1914), Kiyono (1914b), Downey (1922), Maximow (1924, 1927c) stress the identity of the reticulum cells and sinus-lining cells; Bunting (1905), Tschaschin (1914), Maximow (1922, 1924, 1927b), Paschkis (1926), Downey (1915, 1922), Maximow and Bloom (1938), have similarly stressed this point regarding lymph nodes. On the other hand the sinus-lining cell with a distinctive morphology of its own has long been known. Billroth (1861) already speaks of " bekannten, spindelförmigen, gekräuselten Milzzellen, mit seitlich anhängendem, scharfausgeprägtem Kerne, in den Gefässen liegend " as forming the inner longitudinal layer of his venous capillaries. In the older German texts on histology and histopathology it has repeatedly been mentioned and briefly described (see Paremusoff, 1911),usually under the name of " Milzfasern ", or of " Stabzellen " (Weidenreich, 1901). The classical description of Mollier (1910-11) may be mentioned here. Foot (1927) (human spleen), and MacKenzie et al. (1941) (mouse spleen especially) are both in agreement that sinuses and intralobular veins are morphologically identical, the difference being merely one of size. MacNeal (1929) draws a definite distinction between the endothelium of sinus walls and of the smaller splenic veins (*inter*-lobular veins?) into which the sinuses open, in the case of the perfused human spleen.

Regarding the degree of development of the sinus-system, there is also some conflict of opinion. Mollier mentions man, sheep, ox, rabbit, guinea-pig, and dog as having a very extensive system, but Watzka (loc. cit.) places pig, cat, dog, ox, sheep and horse amongst those having a poorly developed system in contrast to man. We may be pardoned for complaining that the diametrical opposition between the views of Knisely (1936a, b) and MacKenzie *et al.* (1941) on the path of the circulation as seen in transilluminated spleens constitutes " the last straw ".

Riedel (loc. cit.) discusses this question, but does not go further than correlating the varied terminology of Billroth (1861, 1862), Weidenreich (1901), Neubert (1922) and Robinson (1930), and covering only the channels concerned in venous drainage. According to Weidenreich (1901), the venous sinuses form plexus-like anastomoses; as such they are seen in the guinea-pig, although fewer anastomoses are shown (*vide* Snook, 1944), but not in the cat (Robinson, 1930). MacKenzie and co-workers who made observations by the transillumination method mainly on mice, but also on rats, rabbits, guinea-pigs and cats, are emphatic that no sinuses exist which are connected at both ends with other sinuses; only laterally placed anastomoses may commonly connect neighbouring sinuses, which belong to the same or different intralobular vein branches, and these anastomoses are *actual or potential channels in the pulp.*

Apart from species differences, there is the important aspect of changing circulatory states of the spleen. The influence of these changes on the histological appearance was pointed out by Mills (1926) and by Hueck (1928); the transillumination experiments brought this aspect into the limelight, both literally and figuratively. This, we feel, is to a great measure responsible for the differences of opinion. If MacKenzie and his co-workers are correct, then the " Zwischenstück " of Thoma is part of the pulp, which, in a contraction phase, temporarily assumes the appearance of an " intactly walled arterio-venous connection ". It is quite conceivable that this space can, in the fixed and sectioned spleen, be mistaken for a sinus. Unfortunately Snook's promised work on the histological appearance of the spleen in different circulatory states has not appeared (or, at least, it has not come to our notice).

We doubt whether in some species sufficient care and attention have been paid to distinguishing " sinusoids " (temporary pulp spaces) lined by littoral cells in a special sense of the term (i.e. reticulum cells temporarily of littoral location) from " sinuses " (venous channels), lined by a distinctive type of endothelium. Embryologically they are of different origin, inasmuch as the pulp spaces are formed in the loose mesenchyme of the dorsal mesogastrium and only secondarily become intercalated in the pre-existent capillary circulation (Thiel and Downey, 1921). These authors state: " The ' sinuses ' (N.B. pulp spaces) are never lined by endothelium; they are merely irregular channels through the reticulum, in the adult as well as in the embryo."

Regarding point (*c*) we wish to refer the reader to Eliot's (1926) work. Foot (1927) makes the pertinent observation that occasionally mononuclears lie in an almost unbroken arch at the periphery of the sinus, just within the endothelium. When numerous, they resemble the endothelial lining, which is swollen. They are easily washed away and do not form an adhering membrane when teased loose. They are probably laggards in the sinus or are entering or leaving through the fenestra. It is on appearances like these that Sabin, Doan, Cunningham (1925) base their conclusions regarding the endothelial origin of the clasmatocyte in the adult. Steudemann (1914) makes the following relevant statement: " Dass die grossen, frei in den Sinus liegenden Makrophagen aus Sinus-endothelien hervorgehen, kann man zwar nicht jedesmal mit Sicherheit behaupten, aber doch als wahrscheinlich annehmen. Sicher ist aber auch, dass man in der Wand der Sinus oft Zellen findet die dafür zu sprechen scheinen, dass, wie Kiyono schon bemerkt, Zellen aus der Pulpa auswandern und nun auf dem Durchtritt durch die Wand der Sinus fixiert sind." Note, too, in this respect Mallory's (1898), figure 33 (plate 61), where his so-called endothelial leucocytes are clustered *beneath* the endothelium of a venous sinus. It is thus scarcely surprising that statements regarding phagocytic activity and haemopoietic potentialities of the sinus endothelium are contradictory.

A few selections from the literature will emphasise that physiological differences between reticulum cells and endothelium of the venous sinuses do exist. As far as phagocytosis is concerned, MacKenzie *et al.* (1941) could not find any trace of phagocytosis of injected india ink particles. Tait and Cashin (1925) found that the endothelium was " lightly peppered "; and that only after heavy injections with india ink. This we interpret as deposition on the endothelium as pointed out by Jaffé (1938), who found the reticular cells to be more active both towards the carbon particles of india ink as well as towards electro-negative colloids. Klemperer (1938) did not illustrate any phagocytosis of thorotrast by endothelial cells (pictured together with phagocytosing reticulum cells and macrophages—his fig. 34), but stated that this occurs " less conspicuously " than by reticulum cells. Versé (1925) in his experiments on cholesterin administration

found the sinus endothelium only partially phagocytosing cholesterin-fats, whilst the phagocytosing reticular cells were crammed full. (Anitschkow, 1914, found little difference in this respect.) Schultze (1912) found lipoid-hoarding only by the reticulum cells in a case of diabetic lipaemia. Pick (1925) derived Gaucher cells from the reticulum cells and from the "adventitial histiocytes", not from sinus cells. He criticizes Epstein (1924), whose illustrations of Gaucher cells originating from the endothelium of the venous sinuses are most unconvincing. It is interesting to note that Pick found strong "haemosiderosis" of the endothelium as shown by the Turnbull reaction for iron. (Lepehne, 1918, casually mentioned that the sinus-cells contain very little iron, which, however, increases after experimentally produced haemolysis.)

MacJunkin (1925a) found only fine red granules in the endothelium of the splenic sinuses of vital carmine-stained rabbits, whereas the mononuclears were conspicuously marked. On the other hand, Aschoff and Kiyono (1913) and Aschoff (1926) ascribed a much greater hoarding ability to the endothelial cells than to the reticulum cells, but they defined the endothelial cell in such a way that it can only mean the reticulum cell in littoral position. Cunningham (1924) also regarded the "endothelial cell" as being more phagocytic, and used this characteristic as a means of distinction. Tschaschin (1914), on the other hand, drew no such distinction between reticulum cells and sinus cells, as regards either their capacity for taking up vital dyes, or their ability to turn into macrophages.

In the haemal nodes of sheep, Woollard and Wislocki (1923) sharply distinguished between venous lacunae (true venous sinuses) lined by inactive endothelium only occasionally taking up carbon particles (injected intrajugularly in the form of india ink) and blood spaces (blood-sinuses) lined by much more actively phagocytosing reticulum cells. Such is the influence of generally held views that these authors supposed, albeit unwillingly and hesitatingly, that the endothelial cells are responsible for the free macrophages seen in the blood spaces.

Taliaferro and Mulligan (1937) were puzzled by the phagocytic inactivity of sinus-lining cells in monkeys with *Plasmodium knowlesi* infection, because, in accordance with the common doctrine, they expected vigorous behaviour of these cells towards foreign particulate matter.

We do not deny that the sinus endothelium may take up some vital stain if dosage is heavy enough, but then so do common vascular endothelium and fibrocytes. It may even be found that these cells occupy an intermediary position physiologically as they do topographically between littoral cells and common endothelial cells.

With regard to possible haemopoietic potentialities of sinus-lining cells, Lang's (1938) emphatic denial of "any cytogenic significance of either the vascular endothelium or of the histiocytic (?) wall cells of the sinusoids of the liver, spleen or adrenal" may be quoted. Consistent with this is Thiel and Downey's (1921) statement: "The endothelium of well-established vessels of the spleen apparently plays no part in the formation of fixed or free cells, or of adventitial cells, about the vessel wall." Sabin and Doan's (1926) postulation of an endothelial origin of clasmatocytes in contradistinction to a reticular origin of the monocyte, does not rest on a very secure basis. Their illustrations (plate 31) do not provide a closely graded series between desquamated endothelium and clasmatocytes. Besides, there was apparently no distinction possible between sinus-endothelium and ordinary endothelium in supravital preparations, nor were they aware of such a difference. Their endothelial cell as stained by Wright's stain, is nothing but a cell from the common vascular endothelium; and those who have upheld the cytogenic potencies of the common adult vascular endothelium have long been

disposed of (*vide* Lewis, 1925, Eliot, 1926, Lang, 1926a, Maximow, 1926, 1927c, Clark and Clark, 1930, Ehrich, 1934, and Bloom, 1938a).

Finally, the morphological concept of the R.E. system as originally outlined (both in the " wider " and in the " narrower " sense) is upset by the results of the research of Chèvremont, as summarized by him in 1945. This author finds that " cholin is the decisive factor of spontaneous histiocytary transformation which exists in cultures *in vitro* of skeletal muscles and of subcutaneous connective tissue ". It is probable that the same or similar substances may be responsible for phagocytic activity of other cell types which occasionally and under certain circumstances have been found to phagocytose, such as epithelial cells (from the urinary bladder, gall bladder, tongue and cornea of the frog—Ishikawa and Shimomura, 1926; hepatic cells, and cells from the adrenal, parathyroids, plexus chorioideus, ectoderm, and giant cells from the placenta—Downey, 1916-17; epidermal cells, alveolar cells from the lung, kidney tubule cells and endodermal cells from the intestine, pigment cells from the retina, " flattened out smooth muscle cells ", and amniotic epithelium of chick embryos—Smith, 1921; etc.). That epithelium can be stimulated to phagocytosis by cholin has been demonstrated by Chèvremont-Comhaire (1945). Chèvremont (loc. cit.) has also indicated the presence of " cellular factors " i.e. factors inherent in the cell itself, which are required before the influence of cholin can express itself.

The R.E. concept as a functional entity has been far too valuable in the past, and will retain too great a value in the future, to be disposed of summarily. One is, therefore, faced with the task of more accurately re-naming and re-defining this remarkable system. Critiques have been forthcoming, but the giving of " a local habitation and a name " has always resulted in an admission of defeat. Jaffé (1938) contented himself with the original name; Mann and Higgins (1938) and Petersen (1935) chose to retain the name of " Histocytes " and " Histiocytary system " respectively. The term " histiocyte " or " histocyte " we feel should only be used in the original Kiyononian sense of the word. " Macrophages " has been favoured as an alternative, but Metchnikoff himself (1887) acknowledged that his " macrophages " could also act as " microphages ", whilst we have proof from observation and from the literature, that " microphages " may be " macrophages ".

In view of the foregoing, any attempt at giving a name that implies a morphological delineation of this system is doomed to failure at this stage. We therefore suggest that the simple term " Phagocytic System " be used. Then, according to the competence of any cell under a definite set of circumstances (i.e. according to the activity of Chèvremont's " cellular factors ", and possibly too, according to the rapidity of digestion of phagocytosed material (" nephrophagocytic action "—Mercier, 1912) ([35]) such a cell could be classified in for instance a first, second or even third order. Thus histiocytes would fall in the first order, fibrocytes in the second or third. Species differences, and differences in response to different endogenous and exogenous ingestible matter could more conveniently be indicated within the framework of such a more flexible system. Similarly changes resulting from newer knowledge could more easily be assimilated.

The term " Reticulo-endothelial ", preferably minus the misnomer " endothelial "—unless one wishes to re-define it in a wider sense as Kyes (1932) has done, or with the substitution of the word " littoral " in lieu thereof, would

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([35]) See also Evans and Scott's, 1921, distinction between clasmatocytes and fibroblasts of connective tissue, not only because of greater activity towards vital dyes, but also because of more rapid " digestion " of the dye particles; and Alexeieff's, 1925a, distinction on similar grounds between " plasmaphages " and " monophages ".

still be used to indicate a definite morphological entity in histology, and its derivations in pathology to indicate abnormal states of that entity. Thus there would be minimal interference with existing usage.

The detailed and more exact classification of the "Phagocytic System" then, would be a task for the future.

SUMMARY.

A. The essence of this work has been to provide a systematic description of the elements and an explanation of the chief artefacts of the bovine spleen smear together with an atlas-guide. Hence no general summary is possible. But attention should be drawn especially to the following points: (1) The most active phagocytosing cell of the bovine spleen is a lymphocyte, termed a a phagocytosing lymphocyte by us, and doubtlessly corresponding to the macrophage, clasmatocyte (smaller type), "splenocyte", and even perhaps the "monocyte" of some authors. (2) Phagocytosis in the spleen includes erythrocytes, leucocytes, and blood platelets. *Thrombophagocytosis* is a very extensive phenomenon in the spleen of bovines, only to be appreciated by the use of refined technique. (3) Monocytes are of infrequent occurrence. *They are derived from reticulum cells,* through an intermediate stage which may be spoken of as a monoblast; but not from lymphocytes. (4) Although ultimately in aged plasma cells distinction between the two types is impossible, *plasma cells may be derived from reticulum cells and monocytes, as well as from lymphocytes.* (5) *The sinus-lining cells have a distinct morphology,* especially in their nuclear characters and their content of iron-containing pigment "haemofuscin" at both poles of the nucleus. *No evidence of phagocytic or cytogenic abilities could be demonstrated.* (6) *The Schweigger-Seidel sheath cells are described in smears and details of their morphology are provided.* The view is advanced that they are of the same nature as reticulum cells as far as reticulin production, mobilisation and phagocytosis are concerned, although differing morphologically from them in the resting state. (7) The nature of certain puzzling epithelial-like elements in routine spleen smears was cleared up by the finding that they are *mesothelial cells of the splenic serosa.*

B. Besides the cells already mentioned, the following elements are briefly described as they appear in the Giemsa-stained spleen smear: endothelial cells, smooth muscle cells, mast cells, eosinophils (their youthful appearance and the presence of Liebreich's α^1 granules are commented upon), neutrophils (mention has been made of their macrophagocytic activity), megakaryocytes, erythrocytes, as well as blood platelets, reticulin, collagen and elastic fibres, haemosiderin and "haemofuscin".

C. *The status and genesis of artefacts* have been discussed. Theoretically a distinction can be drawn between artefacts produced by making and subsequent manipulation of preparations (exogenously produced artefacts), artefacts conditioned by states of senescence or degeneration of the cells (endogenously conditioned artefacts), and artefact-like appearances resulting from physiological processes; in practice this distinction is not always possible. The artefacts described include cellular distortion, cytoplasmic fragments and free cytoplasmic constituents, naked nuclei, distorted and smudged nuclei, various contaminants, stain deposit, etc. The effects of autolysis have also been mentioned.

D. Problems of *cell derivation and terminology* have been discussed.

E. In view of the phagocytic powers of lymphocytes and neutrophils and the inactivity of the sinus endothelium in this respect, *a critique is offered on the R.E. system,* and the proposal is put forward that the name be changed to "Phagocytic System".

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DESCRIPTION OF PLATES.

All the illustrations were sketched by aid of camera lucida, using a Leitz 1/12 oil immersion objective (N.A. 1.30) compensating Zeiss ocular K 7x and condenser 1.4., oil immersed. The actual final enlargement is x 1,330.

PLATE I.

Lymphocytes.

Fig. 1.—Small lymphocyte.

Fig. 2.—Medium lymphocyte.

Fig. 3.—Large lymphocyte (" pro-lymphocyte " or " lymphoblast ").

Fig. 4.—Immature lymphocyte (" lymphoblast ").

Figs. 5 and 6.—Medium lymphocytes containing fine and coarse azur granules respectively.

Fig. 7.—Binucleate lymphocyte.

Fig. 8.—" Rieder cell ". (Medium lymphocyte with lobed nucleus).

Fig. 9.—Mitotic lymphocyte (anaphase) from the peripheral blood of a calf obtained during immunization to Babesiosis and Anaplasmosis. (See Text page 91.)

Fig. 10.—Lymphocyte with voluminous, very pale staining cytoplasm. The thickening of the edge of the cell is an artefact.

Fig. 11.—Erythrophagocytosis by a lymphocyte.

Figs. 12 to 14.—Thrombophagocytosis by lymphocytes.

Fig. 15.—Thrombophagocytosis in a cell type intermediate between reticulum cell and large lymphocyte.

Figs. 16 to 18.—Simultaneous erythro- and thrombophagocytosis by lymphocytes.

Fig. 19.—Leucophagocytosis by a lymphocyte.

Fig. 20.—Haemosiderosis of a lymphocyte.

Fig. 21.—Lymphocyte: haemosiderosis and platelet phagocytosis.

PLATE I.

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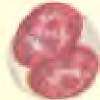
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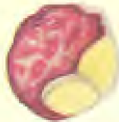
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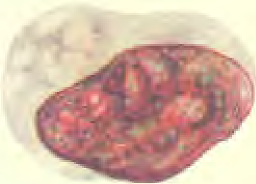
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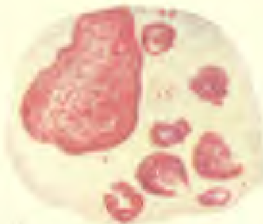
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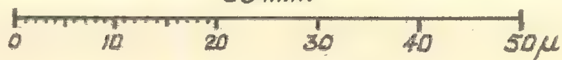

PLATE II.

Plasma cells.

Fig. 22.—Small plasma cell.

Fig. 23.—Medium plasma cell.

Figs. 24 and 25.—Large plasma cells. Note the different phases of maturity of the nuclei.

Fig. 26.—Medium sized plasma cell still in the transition phase from a lymphocyte.

Fig. 27.—Plasma cell derived directly from reticulum cell.

Fig. 28.—Plasma cell development in a " monoblast ". (This cell was found in a preparation showing plasma cell development from reticulum cell—monocyte lineage.)

Fig. 29.—Plasma cell with minute vacuoles.

Figs. 30 and 31.—Transitions of vacuoles to Russell bodies.

Figs. 32, 33, 34.—Russell body plasma cells.

Figs. 35 and 36.—Plasma cells containing thrombocytes.

Fig. 37.—Plasma cell containing red-staining (metachromatic) material.

Figs. 38-39.—Haemosiderosis of plasma cells.

Fig. 40.—Plasma cell with " S "-shaped nucleus. Note diplosome and negative Golgi image.

Figs. 41 and 42.—Binucleate plasma cells.

Fig. 43.—Binucleate plasma cell apparently resulting from amitosis.

Fig. 44.—Trinucleate plasma cell (of megakaryoblastic derivation?) Note centrosome.

Fig. 45.—Quadrinucleate plasma cell (of megakaryoblastic derivation?) Note centrosome.

Figs. 46-49.—Stages in ageing and degeneration of plasma cells, fig. 49 illustrating a " Jolly body plasma cell ".

Fig. 50.—Plasma cell with karyorrhectic nucleus (= Amitotically dividing pyknotic nucleus?) Note this cell fits in well in the series of degenerating cells, viz. between figs. 46 and 47.

Fig. 51.—Mitosis of plasma cell.

PLATE II.

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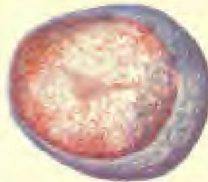
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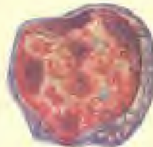
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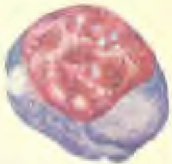
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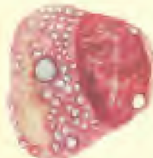
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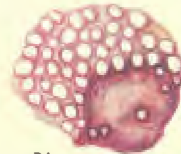
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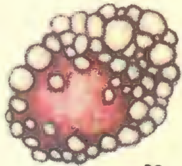
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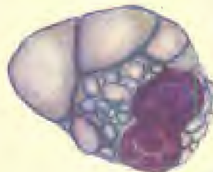
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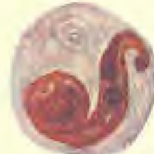
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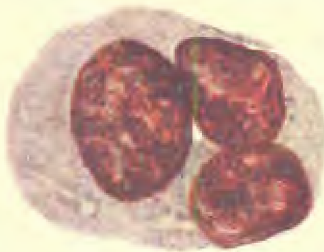
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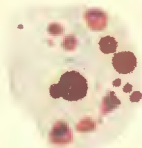
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PLATE III.

Reticulum cells.

Fig. 52.—Reticulum cell.

Fig. 53.—Binucleate reticulum cell.

Fig. 54.—Haemosiderosis of a reticulum cell.

Fig. 55.—Erythrophagocytosis by a reticulum cell.

Fig. 56.—Thrombophagocytosis by a reticulum cell. (Note intranuclear position of one platelet).

Fig. 57.—Mobilized reticulum cell. Compare nuclear texture with that of monoblast derivative (fig. 62) and with peripheral sheath cell (fig. 67).

Transitions from Reticulum cells; Monocytes.

Figs. 58-59.—Developing monocytes from reticulum cells, i.e. "monoblasts". Fig. 59 has "pro-monocyte" type of nucleus, but cytoplasm is still immature. Note diplosome.

Fig. 60.—Monocyte.

Fig. 61.—Monoblast. Note relatively mature state of cytoplasm and well defined diplosome.

Figs. 62 and 63.—Monoblast derivatives containing haemosiderin.

Fig. 64.—Monoblast derivative containing platelet material.

Sheath cells.

Fig. 65.—Group of Schweigger-Seidel sheath cells. Note nuclear texture, very prominent centrioles and intimate association with reticulin fibrils.

Figs. 66 and 67.—Sheath cells—peripherally lying, mobilized types.

Fig. 68.—Sheath cells, containing granular material resembling platelet chromomeres. (Compare with the group of platelets lying at lower left hand corner).

PLATE III.

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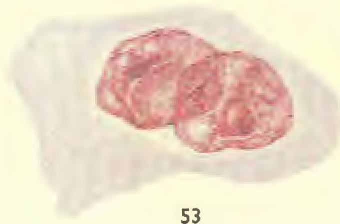
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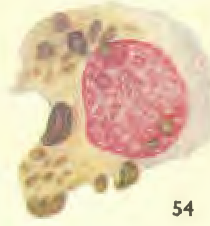
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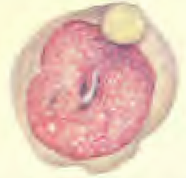
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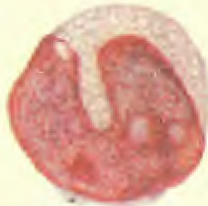
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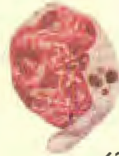
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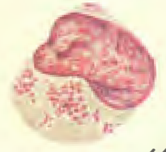
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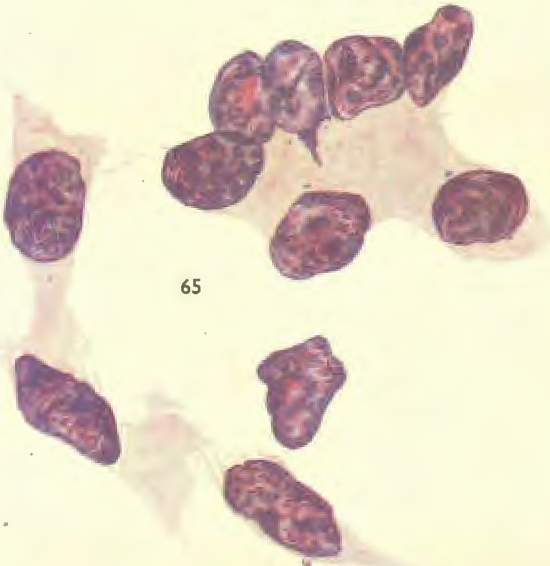
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PLATE IV.

Endothelial cells.

Fig. 69.—Endothelial cells of capillary. Note contained red cells and fine neutrophilic granules.

Sinus-lining cells.

Fig. 70.—Sinus-lining cell. Note haemofuscin and neutral-staining granules.

Fig. 71.—Sinus-lining cell. Nuclear texture not typical. In our opinion this type of cell is of a character intermediate between sinus-lining cells and those of the common vascular endothelium.

Smooth Muscle cells.

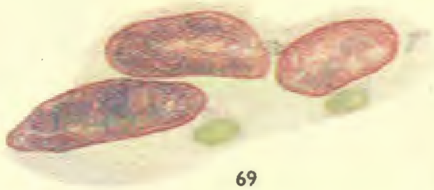
Fig. 72.—Smooth muscle cell.

Fig. 73.—Group of smooth muscle cells, closely associated with reticulin fibres.

Serosal cells.

Figs. 74-77.—Mesothelial (serosal) cells. Note "C"-shaped nuclei (fig. 76) and doughnut-shaped nuclei (figs. 74 and 77) and also the presence of exoplasm and endoplasm (fig. 75).

PLATE IV.



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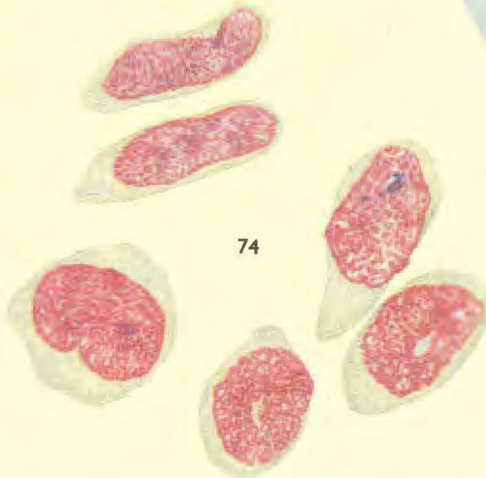

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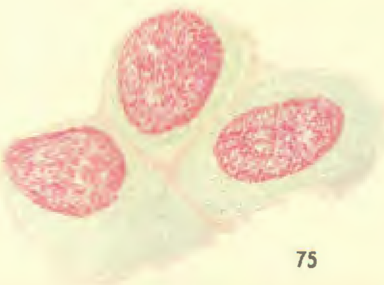

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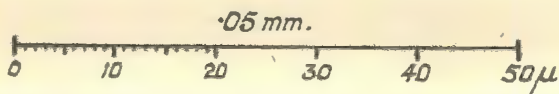

PLATE V.

Miscellaneous cells; Thrombocytes.

Figs. 78-79.—Mast cells.

Figs. 80-81.—Eosinophils. Note Liebriech's α^1 granules (fig. 81) and also the cytoplasmic immaturity (incomplete filling with granules).

Figs. 82-83.—Neutrophils containing (fig. 82) phagocytosed platelets and (fig. 83) a phagocytosed red cell.

Fig. 84.—Megakaryocyte.

Fig. 85.—Erythrocytes, showing usual range of colour reaction with Giemsa.

Fig. 86.—Thrombocytes.

Connective tissue fibres.

Fig. 87.—Mass of reticulin fibrils.

Figs. 88-89. Elastic fibres.

Fig. 90.—Bundle of collagen fibrils.

Pigments.

Fig. 91.—Haemosiderin (free-lying).

Fig. 92.—" Haemofuscin " (free-lying).

PLATE V.

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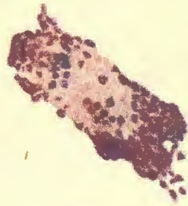
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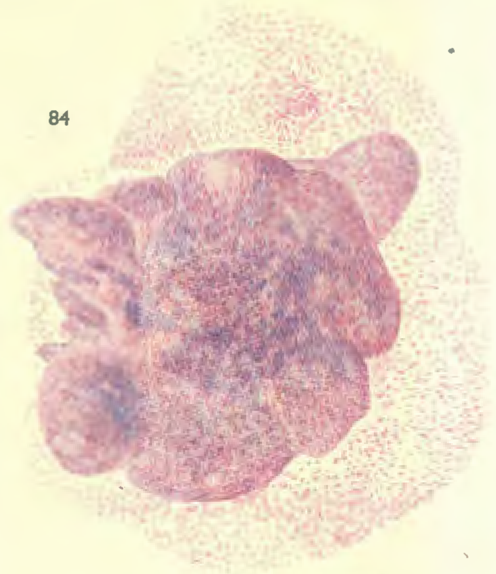
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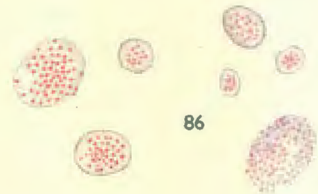
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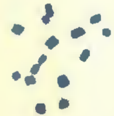
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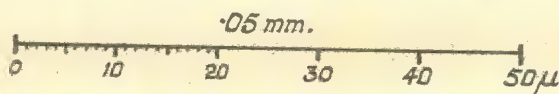

PLATE VI.

Artefacts.

Fig. 93.—Globules of free cytoplasm derived from lymphocytes and resembling red cells.

Fig. 94.—Free lymphocyte cytoplasm containing a thrombocyte and vacuoles.

Fig. 95.—Free lymphocyte cytoplasm containing fine and coarse azur granules. (Compare with Koch's bodies).

Fig. 96.—Free cytoplasm containing a phagocytosed red cell and platelet chromomeres.

Fig. 97.—Free cytoplasm containing thrombocytes with chromomeres of varying coarseness. (Note resemblance to Koch's bodies).

Fig. 98.—Free cytoplasm containing a thrombocyte and a centrosphere with diplosome.

Fig. 99.—Free cytoplasm containing Russell bodies.

Fig. 100.—Free megakaryocyte cytoplasm. (Note diplosomes).

Figs. 101, 102, 103.—"Naked" nuclei from small, medium and large lymphocytes respectively. The latter still shows wisps of torn off cytoplasm.

Fig. 104.—Free reticulum cell nucleus.

Fig. 105.—Free megakaryocyte nucleus.

Fig. 106.—Free sinus-cell nuclei. Note adherent "haemofuscin".

Fig. 107-108.—Nuclear smudges.

Fig. 109.—Free nucleus superimposed on a nuclear smudge; (an "artificial cell").

Fig. 110.—Chromatin filament drawn out from a nucleus.

Fig. 111.—Network of chromatin filaments from smudging of nuclei.

Fig. 112.—Chromatin filaments snapped off and sometimes recoiled. Note resemblance to *B. anthracis*.

PLATE VI.

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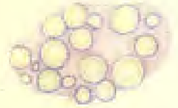

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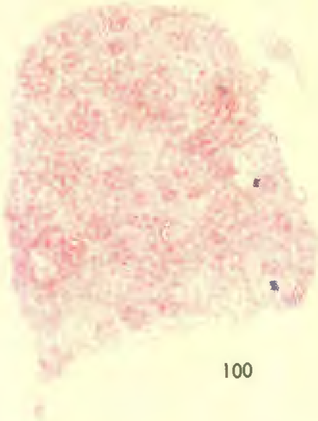

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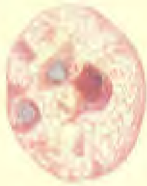

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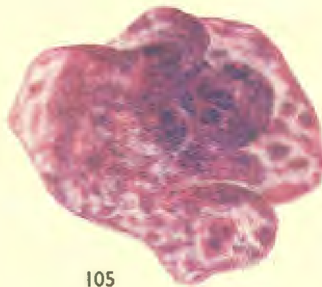

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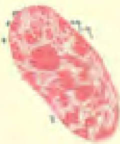

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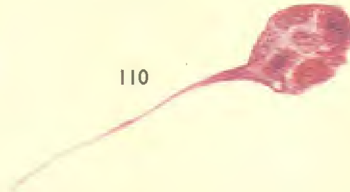

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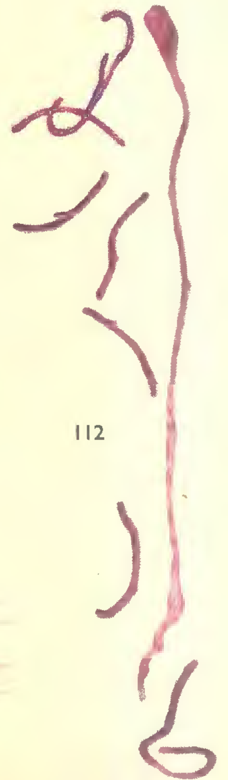

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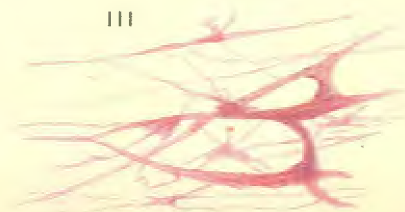

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PLATE VII.

Artefacts (continued).

Fig. 113.—Chromatin globules. Note formation from nucleus and admixture with cytoplasmic masses.

Figs. 114-115.—Free eosinophil granules (cf. Koch's bodies). Some are superimposed on red cells.

Figs. 116, 117, 118.—Free mast cell granules (in figs. 117 and 118 superimposed on red cells).

Fig. 119.—Free Russell body.

Fig. 120.—Vacuoles, one partially superimposed on a lymphocyte nucleus.

Figs. 121-122.—Vacuoles associated with lymphocyte nuclei. (See text page 110).

Fig. 123.—Contaminating cocci.

Fig. 124.—Contaminating oöspore (?). (See text pages 110 and 111).

Fig. 125-127.—Fungus spores (see footnote 29, page 111).

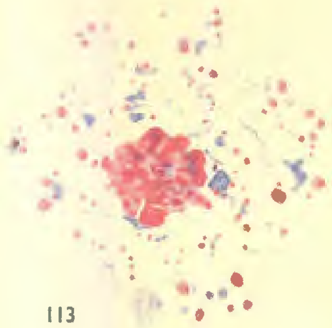
Figs. 128-130.—Various types of stain deposit. In fig. 130 restricted to red cell surface (cf. punctate basophilia).

Fig. 131.—Effect of light scratches over nucleus of a stained cell.

Fig. 132.—Autolysing nuclei.

Fig. 133.—Phialide of a mould (?) (See text page 111).

PLATE VII.



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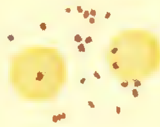

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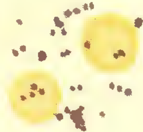

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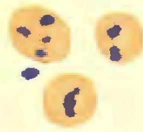

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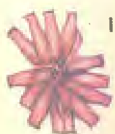

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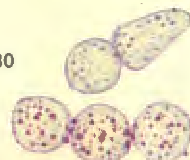

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