A Review OF THE STRUCTURE AND FUNCTION OF THE SPLEEN.*

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I WISH to say what a pleasant thing it is for me to be back in a familiar lecture-room under the presidency of my distinguished chief and teacher of physiology. When in 1899 as a young student I attended the first course of lectures that he gave as professor in Edinburgh—which I still look back upon with delight—he told us, along with other wonderful things, about the known attributes of, and about the perplexities that surround, the function of the spleen. As happened so often in his case, the information he gave was from first-hand knowledge, for he was fresh from an important investigation on the subject, which had taken a winter of work by his pupil Benjamin Moore and himself. That work was fundamental for our knowledge of the muscular and nervous action of the spleen. I wish I could go on to say that there and then I was filled with an eager desire to probe further into the mystery that shrouded splenic function. I cannot speak for my classmates, but certainly nothing was further from my thoughts. At the same time, it was of distinct value to have had one's introduction to the spleen from a master who had equal range over the finer structure as displayed by the microscope and over the gross behaviour of the organ as studied under the conditions of instrumental laboratory investigation.

My particular interest in the spleen began accidentally, when Miss Macnaughton (now Mrs. McCartney) and I were engaged in injecting particles of Indian ink into the circulation of mammals, so as to compare with some of the results previously obtained by similar injection into Crustacea. We found that the particles, even when injected in enormous quantity, were speedily swept out of the circulation and deposited in the spleen, in the liver, and in the bone marrow. While the foreign particles slipped past the cells of the vascular endothelium, without any tendency to adhere to them, they had in the liver, spleen, and bone marrow been caught and ingested by special cells that here line the blood channels. In other words, in each of these three organs of the mammal there are naked areas devoid of the normal endothelial lining, and many of the cells situated along these bare areas are phagocytic. We thought for the moment that we had lighted upon a new fact of obvious importance in connexion with blood spread of infections—malaria, typhoid, kala-azar, small-pox, tuberculosis. Our teachers in discussing these diseases had certainly told us little of the extraordinary phagocytic properties of these cells in spleen, liver, and marrow. As so often happens with discoveries so simply and so easily made, other people had been in the field long before us, only their work had been forgotten. We were also unaware that Aschoff had, just before us, seized the great significance of the same facts.

This was in the early days of the war. The next thing was that I found myself out in Macedonia treating malaria patients in the actual country from which we obtained our word spleen, and where, 2,500 years ago, this organ commanded almost as much attention—in connexion both with sacrificial ceremonies on animals and with disease in man—as the brain or the heart does in modern times with us. To the early Greek physicians—and Galen later reflects the same outlook—the curious variations in volume of the malaria-infected spleen possessed an interest that we more fortunately situated people find difficult to understand. So prevalent has malaria long been in Greece that there is reason to believe that this disease, even more than political dissension, proved the ultimate undoing of the country.

* A paper read in opening a discussion on this subject in the Section of Physiology and Biochemistry at the Annual Meeting of the British Medical Association at Edinburgh. Professor Sir Edward Sharpey-Schafer, M.D., F.R.S., President of the Section, was in the chair.

It was an arresting experience to come back from Greece and from Macedonian "Erlebnisse," and to read the *fons et origo* of medical literature, Hippocrates, where the ravaging story of malaria stands printed on page after page of the most original, the most detached, and objective writing in the whole of medicine. Had we not had unusual medical experience in Macedonia, for which our clinical training, our pathology, had failed to prepare us—cases of black urine, cases of delirium, of coma, bodily disorder protean in variety and baffling as regards both rationale and treatment? And in our ignorance we had imagined that these manifestations of disease were being seen and recorded by us for the first time in history. Some of you may in your childhood's days have landed on some lone deserted island. As flocks of wild birds rise on the approach of your boat you imagine that you are actually a Robinson Crusoe, setting foot for the first time on utterly unknown shores. When you get older you discover that your Crusoe island has a proprietor, has been carefully mapped, measured, and charted, has even had its human population in earlier days, to whom every beach, every curve of the coastline, every jutting rock, was perfectly familiar. So it was on returning from Macedonia to read the story of malaria in Hippocrates.

In the course of this disease Hippocrates finds that the spleen may suddenly enlarge, and within a day or two become again reduced in size. One of his remarks, transmitted by Galen, is, *δοις γε τὸ σῶμα θάλλει, τοῖς δὲ σπλὴν φθίνει*—that is, when the body flourishes the spleen wastes. We examined the children, often anaemic and ill nourished, of the villages near our camp. We found spleens reaching down to or below the level of the umbilicus. In other words, the enlarged spleen represented the normal condition of the Greek child, whence the point of Hippocrates's unusual remark. Could any statement better illustrate the universal prevalence of malaria in his day? Galen informs us that both Hippocrates and Plato considered the spleen as an organ for purifying the blood, an opinion as prophetically consistent with modern findings as are the atoms of Democritus. If we can make one single all-embracing statement about the spleen as it occurs throughout the range of the vertebrates, it is that it purifies or "catharses" the blood.

However, this is not an historical lecture. I have to open a discussion in the year 1927, in the Physiology Section of the British Medical Association, and under the exacting eye of Sir Edward Sharpey-Schafer himself. There are other speakers too—for instance, Professor Barcroft—whose work on splenic function has been so fruitful and so illuminating.

FUNDAMENTAL FEATURES.

The spleen is essentially a vertebrate organ, and its steady persistence throughout the group is an indication of its importance. Comparative study shows that it has certain common structural features, which we may count fundamental or essential. In some animals—for example, mammals—we find additional structures, notably lymphoid tissue and splenic muscle; these we may regard as secondary acquisitions. Let us devote our attention to the fundamental features, which may be studied to particular advantage in the spleen of the skate.*

The Spleen of the Skate.

This is a tough and freely movable organ, externally quite like the spleen of mammals. It has, however, no lymphoid tissue, no trabeculae, and no special splenic musculature, the only muscle within it being that which invests the arteries. The firmness of the organ is due chiefly to the strong-walled vessels that ramify within it. These provide a tough, stringy foundation, which in turn is embedded in a more deformable, more dilatible reticulum. The fibres of this reticulum, which serves to enmesh all the cellular elements of the parenchyma, are stouter, stronger, and more conspicuous than those met with in any mammalian spleen, yet their strength, as dissection shows, falls far short of that of the ramifying arteries and veins. In other words, so far as mechanical resistance is concerned, the principle of construction in the skate spleen is like

* The anatomical details here given in regard to the skate spleen have been worked out in conjunction with Dr. E. S. Mills.

that employed in ferro-concrete, where strings or rods of more resistant material greatly augment the strength of the composition. A fibrous capsule surrounds the whole.

Development.

As Laguesse has shown, the spleen of elasmobranchs as well as of other fishes takes origin as a kind of reticulated venous sinus alongside the subintestinal (later the portal) vein, with which it stands in open communication. At an early stage no regular or rapid blood current sets through it, though delicate ramifying sinuses begin to form defined channels for blood eddies. Arteries eventually penetrate from the exterior into this spongy, sinus-provided tissue. When they have worked their way well into the interior their ends become open, and then for the first time arterial blood pours into the reticulum. As might be expected, the strong-walled veins proper of the organ have been taking form and semblance during the time of inroad of the arteries, and now for the first time the natural through-and-through current is established. There are thus two distinct stages in the development of this unique organ, and even after the second stage the circulatory arrangements remain unusual. In the first stage the spleen is a spongy, blood-sodden, yet sinus-provided excrescence on the side of a vein. In the second stage arterics, carrying their muscle coat and their fibrous adventitia with them, shoot in from the exterior. Coincidentally, well formed veins, with fibrous tissue wall, reach inwards as a counterpart to these arteries. Though they trend towards each other their terminations never meet by direct inosculation. Everywhere between the ends of the tubular arteries and the stout-walled veins lies a marsh, a morass of reticulum with its runnels, the venous sinuses.

In this description I have closely followed Laguesse. At one point only have I ventured to modify his account. He would have it that the walls of the intrasplenic veins are everywhere formed of compacted reticulum. In point of fact, over a considerable part of their intrasplenic course the walls of the larger veins are composed mainly of tendon-like bundles of wavy, non-branching, white, collagenous fibrils, which may be assumed to have grown in from the adventitia of the parent vein or veins. The spleen of the skate, therefore, consists of arteries which have invaded its texture; of veins, which we may also look upon as having grown in from the exterior; and between these two—what? The original reticulated and sinus-provided sponge which originated in close relation to the subintestinal veins.

Arteries and Ellipsoids.

The arteries eventually terminate in penicillar vessels. Each penicillus gives off near its extremity a small cluster of terminal vessels. Each of these in turn ends in one or more ellipsoids. The ellipsoidal mode of termination of the arteries is a fundamental feature of splenic architecture, and in fishes, just as in mammals, the sessile phagocytic cells of the ellipsoids capture foreign particles introduced into the blood stream and probably also ingest effete corpuscular elements of the blood, this being our modern conception of the "blood-cleansing" process of Hippocrates. It is noteworthy that in the spleen of the skate each terminal vessel comes to a blind end within the ellipsoid. In the mammalian spleen the terminal vessel frequently passes through an ellipsoid and ends beyond it. Whether this peculiarity of the arterial endings in the mammalian spleen is correlated with the presence of the special splenic musculature I am unable to say. The spleen of the skate, which is devoid of muscle, exercises no detectable pumping action, and it is a debatable question whether it acts as a temporary store of erythrocytes as Barcroft has shown the mammalian spleen to do. In texture the ellipsoids resemble those of the mammalian spleen, being composed of very finely meshed reticulum, which is continuous with the general reticulum of the parenchyma.

Veins and Venous Sinuses.

The veins of the skate spleen originate as venous sinuses, this mode of origin being again a fundamental splenic feature. The sinuses have none of the elaborate architecture described by Mollier in the case of the mammalian

spleen. They are mere endothelium-lined tunnels in the general reticulum. Although to the eye the lining sheet of endothelium appears continuous, the blood and blood corpuscles can evidently pass freely between the endothelial cells, for nowhere is the uniform continuity of any sinus wall seen to be broken. As in the mammalian spleen, the walled veins do not reach quite so far into the organ as do the arteries, the place of the penicilli being taken by venous sinuses. The disposition of the veins, however, is different from that in the mammalian spleen, in which these vessels are carried by trabeculae. In the spleen of the skate, which is devoid of trabeculae, venous and arterial branches tend to run side by side, being bound together in a common investment of thickened reticulum. Within this common reticular sheath the larger venous branches have a very stout wall composed of bundles of non-branching collagenous fibrils. In possessing a real fibrous tissue wall both the arteries and the veins of the spleen may be regarded as foreign intrusions lying in the midst of the general reticulum. The venous are invariably of wider calibre than the corresponding arterial branches.

Fundamental Vascular Features.

Let us now review the vascular arrangements, confining our attention solely to the fundamental features. Inasmuch as the arteries and veins have a wall containing real fibrous tissue they constitute intrusions into the splenic reticulum. The arteries are a little longer than the veins; at the level at which the arteries ramify into penicilli the veins give place to venous sinuses. The penicilli break into terminal vessels, each associated with one or more ellipsoids, which are composed of finely meshed reticulum. The ellipsoids contain sessile phagocytic cells, which, after ingestion of foreign particles, migrate into the parenchyma. Within an ellipsoid the endothelial lining of the arteries may come to an end. Between the ellipsoids and the venous vessels intervenes the general reticulum of the spleen. The blood passing through this reticulum enters venous sinuses before reaching the veins proper. Notwithstanding the apparent continuity of the lining endothelium of the venous sinuses, their walls are everywhere porous. The venous are of larger calibre than the corresponding arterial branches.

The Reticulum.

The reticulum of the skate spleen is beautifully displayed by the Bielchowsky staining procedure. Fortunately, too, the Bielchowsky method in this case usually makes a distinction in depth of colouring between the true reticular and the non-branching collagenous fibres present in the wall of the veins and arteries and in the external capsule. Thus the limits of the reticulum can be defined, and we shall devote our attention to the problem of assigning its exact boundaries.

Besides the Bielchowsky method of displaying the reticulum there is another equally instructive method, which serves, too, as a valuable check upon the staining procedure. This is to inject backwards through the main splenic vein a suspension of Indian ink particles, followed by a similar injection of formalin. The latter material, clearing the ink out of the veins and venous sinuses, drives the last of it into the reticulum, which the reagent also fixes—and here I might say that, whatever its disabilities for fixing cells, in its application both to reticular and to white connective tissue fibres, formalin for special reasons stands supreme as a fixing agent. The particles of ink find their way to every available part of the reticulated interior, and as the reticular fibres have as strong a molecular attraction for these particles as the strands of a cobweb have for dust, our microscopic section after this form of treatment shows practically the full extent of the ink-laden cobweb of reticulum. Instead of dyeing or staining, we have here succeeded in painting the reticular fibres, which is a rather unusual histological procedure. There is one exceptional region. The ink does not gain entry to the ellipsoids, which therefore remain unpainted. To coat the reticular fibres of these structures we must inject the pigment, not by way of the veins, but by way of the arteries. By what means this sharp barrier is maintained at the surface of the ellipsoids is hard to explain.

The Reticular Boundaries.

Let us now consider the real reticular boundaries. When we remember that over and above the fibrous capsule, on which the reticulum must of necessity demarch, there are only two major intrusions into the tissue—namely, the arteries and veins—it is plain that the ultimate boundaries of the reticulum are either capsular or vascular. Suppose we examine the reticulum where it encounters one of the larger arteries or veins. As they come progressively within the sphere of influence of these structures the interlacing reticular fibres uniformly and rapidly increase in thickness. While the fibres thus enlarge their meshes rapidly become smaller, until finally, in contact with the true fibrous material that forms the outer wall of the vessels, the stout reticular fibres have plaited themselves into a close basketwork, in which chinks and interstices are virtually eliminated. Up to a certain point the ink can penetrate between and settle upon the withies of this basketwork, but its further progress seems to be barred by the mere compaction of the reticular material, and just beyond this boundary line lies the true fibrous wall of the vessel. Similar matted condensations of the reticulum may be found at many regions under the capsule, and were this heavy compaction of stout-fibred reticulum present at every part of the boundary surface we might have concluded that the fibrous tissue was separated from the reticulum solely by an intimate intertwinning of thickened reticular fibres. In many places, however, particularly round the penicillar and terminal vessels, but also under the capsule, the reticulum that abuts upon the non-reticular tissue is no more condensed than that surrounding the ellipsoids. Yet the demarcation between the vessels or capsule, as the case may be, and the reticulum is perfectly sharp. Where anchoring strands of reticulum pass radially to the vessels, or normally to the capsule, the various bays or bights between the anchoring fibres are seen to be fringed with black. In other words, a thin but complete screen of reticulum closes off the capsule and the intrusive vessels from contact with the splenic blood.

Two Modes of Arrest of Haemorrhage from an Artery.

In order to appreciate the significance of this arrangement let me digress for a moment to consider an experiment which is easily carried out, though I have not seen it described. Suppose the carotid artery of a dog has been laid bare with the intention of observing the method of arrest of haemorrhage when the vessel is punctured. If the adventitia is removed before puncturing the artery, the blood sprays out for a very long time. When the haemorrhage eventually ceases the wound is seen to be plugged with a platelet thrombus. Coagulation plays no part here in stopping the bleeding. If, on the other hand, the intact vessel is punctured, the course of events is totally different. No external bleeding occurs. The blood simply enters the meshes of the adventitia, which it distends like a sausage. The bleeding is very soon over. No plug of platelets has this time formed in the orifice in the media. Arrest is due to the prompt coagulation of the blood in the adventitia. The same experiment may be carried out on the aorta of a skate. If the adventitia has been previously removed, the wound is eventually plugged by agglutination of the nucleated spindle cells. If the vessel is punctured through the adventitia, prompt coagulation occurs, which leads to rapid arrest of haemorrhage.

Haemothigmic and Anaemothigmic Tissues.

This experiment teaches us that in contact with the fibres of ordinary connective tissue blood undergoes a change, being inevitably induced to coagulate. We know that the blood may come freely in contact with reticulum without being induced to clot. In its negative action upon the blood, reticulum, like ordinary endothelium, may be termed *haemothigmic*—that is to say, these tissues may freely touch blood. By contrast with these haemothigmic structures probably the great mass of the other tissues of the body, and certainly ordinary connective tissue, are *anaemothigmic*; these may not touch blood. In the spleen the anaemothigmic structures—namely, the capsule, the blood vessels, the Malpighian corpuscles, which lie in the

adventitial sheaths of the arteries, and the trabeculae—are screened off by means of haemothigmic reticulum from the reticular interior.

Continuity of Reticular and White Fibres.

A striking fact is that the reticular fibres are structurally continuous with the fibrillar bundles of collagenous material that form the capsule and the true sheaths of the vessels. For a time I was inclined to think that the distinction between the two kinds of fibres, the anastomosing reticular and the non-branching "areolar," is absolute. Our president, Sir Edward Sharpey-Schafer, had observed in lymphatic glands complete structural continuity of the two kinds. It was difficult at first to confirm this in the skate, but renewed examination eventually showed that the larger reticular fibres around the vessels and under the capsule directly and abruptly change into fibrillar bundles of the ordinary white connective tissue type. This is not the place to enter into a discussion as to the possibility of an ultimate chemical separation between the two kinds of material. Hitherto no satisfactory chemical differentiation has been made, and Mallory has recently shown that the supposedly specific reticular stains fail to differentiate between reticulum and ordinary connective tissue fibres. Suffice it to say that there is a difference of a physico-chemical kind, which, involving the surface properties of the fibres, may well lie at the root of their ultimate divergence in features of structural character.

Junction of Endothelium with Reticulum.

Our survey of frontiers is not yet wholly complete, and the "boundary commission" into which this meeting has temporarily constituted itself has still two thorny problems before it. These, being questions of "corridors" and seaports rather than of land frontiers, are of very particular delicacy, and before concluding we must have some decision, however provisional, regarding these critical regions. The blood is conveyed to the spleen in a conduit of endothelium. It leaves the spleen by a similar conduit. The arterial endothelium creeps inwards to a certain distance and then stops. The venous endothelium of the organ, derived from the parent stock of endothelium that lines the chief veins, likewise creeps inwards as far as it may. Between these two conduits of haemothigmic endothelium intervenes the whole irregular sponge of haemothigmic reticulum. How is junction of endothelium with reticulum effected at the two terminal stations respectively?

I have said that the substance of the ellipsoids consists of very finely meshed reticulum. Their central cavity is lined with endothelium. The junctional station in this case is therefore to be sought in the interior of the ellipsoid, and just at the place where the endothelium rests upon the finely meshed reticulum. Although to the eye this endothelium appears to form a complete lining to the central cavity, we know that the blood can pour between its constituent cells. May it be that endothelial cells, possessed of bipolarity as we know them to be, and resting upon a haemothigmic instead of an anaemothigmic tissue, have difficulty in obtaining a proper base or foundation for their attached face? Some such supposition as this is rendered the more likely when we turn from the ellipsoids to consider the venous sinuses, which are also endothelium-lined. In the spleen of the skate these sinuses can be closely studied. They consist of nothing but tubes of endothelium surrounded by the simplest possible reticulum. So far as any special modification of the arrangement of the investing reticulum goes, the venous sinuses might simply have been punched out of the general reticulum with a sharp cheese-borer. Yet when the inner sinus-directed surface of the reticulum encompassing each sinus is examined with a high power innumerable fine brushes of freely ending reticular fibres are found projecting towards the endothelium, which in turn appears in section to form a complete lining to the sinus. Now we know that blood can pour as easily through the wall of these sinuses as through a sieve. The apparent continuity of the lining endothelium is wholly deceiving, and we connect the anomaly of the porosity of the lining with the intrinsic difficulty of attaching endothelial cells to reticular fibres. They are here cemented, not to the haemo-

thigmic sides of the fibres, but to the delicate truncated or amputated ends of the microscopic brushes, which alone can obtain a grip on these pavement cells. This at least is my interpretation, conjectural, but I hope reasonable, of what I see.

CONCLUSION.

In the survey I have elected to deal with one single aspect of the spleen problem. It is true that members of my laboratory in McGill University—Dr. M. F. Cashin, Dr. E. S. Mills, Dr. J. G. P. Cleland—have recently been publishing or participating in publishing certain novel findings with regard to the spleen, but as these are already available in printed form I thought it better to branch away from the details of their work and to attempt to express some general ideas which emerge in considering the peculiar architecture of the organ. There has been no time as yet to put these views, in so far as they are hypothetical, to direct experimental test, or otherwise to develop experimentally any of the further issues that forthwith present themselves.

In view of the increased interest in reticular tissue since the introduction by Aschoff of the term "reticulo-endothelial system," may I be permitted to say that, important as the conception of this system is, the name that Aschoff choose for it is rather apt to mislead, for the reticulo-endothelial system is essentially devoid of any endothelium. In view also of a common misconception attaching to the name, it is well to remember that reticulum occurs in many places to which the corpusculate fluids, blood and lymph, have no access; in other words, the presence of reticulum in an organ does not mean that the organ constitutes part of the reticulo-endothelial system. It is true that reticulum, at least as we find it in spleen, bone marrow, and lymph glands, is haemothigmic; but this may be a mere accident of its constitution, in the same sense as one might say that the excitability of nerve fibres to an external stimulus applied anywhere along their length is a fortuitous rather than a necessary feature of their organization. Should inquiry be made as to the primary function of reticulum as compared, say, with areolar tissue, I should like to make one significant observation. When we examine the various localities in which areolar tissue occurs—for example, between muscle fibres or tendon bundles, under the skin, around nerves, blood vessels, etc.—we find that they are all places where shearing or sliding movements occur. Such movements of slip also occur between serous membranes and in synovial joints. The shearing movements that occur where areolar tissue is found are, however, subject to definite limitation. It is the inextensible white fibres of the tissue that impose a limit on the amount of shearing displacement, the elastic fibres serving the purpose of restoring the structures to the neutral position. Now reticulum constitutes the connective tissue framework of solid glands, such as the kidney liver, or spleen, where shearing displacements as such are not called for, but where uniform expansile movements must be provided for. According to this conception the primary purpose of reticulum is a mechanical one; its haemothigmic properties are secondary.

DISCUSSION.

Professor J. BARCROFT (Cambridge) reviewed the relation between the nervous system and the vascular function of the spleen. Using the preparation in which by a surgical operation the spleen could be placed outside instead of inside the dog's body, it had been shown that during muscular exercise the discharge of the spleen was controlled by the nervous system. The spleen anticipated exercise, and even expelled its blood into the general circulation when activated by jealousy or anger. In the case of a jealous animal, the spleen of which had been made to grow outside it like a mushroom, the organ might be seen to grow pale when the animal's rival was fondled. Was this pallor specific to the spleen, or did it affect the whole alimentary tract? The spleen circulated the blood in its pulp: this also seemed to be ruled by the nervous system, for it did not occur in denervated portions of the spleen. The function of the rhythmic contractions of the spleen might be the production of this concentration.

Professor P. T. HERRING (St. Andrews) pointed out the necessity of considering the allied glands, especially haemolymph glands, in investigating the functions of the spleen. He considered the circulation of blood through a lymph gland and mentioned that cells could be taken up from the circulation and held by the reticular cells. These cells became detached and migrated to the centre of the nodule. It was considered that attention should be paid to this aspect in relation to the lymphoid tissue of the spleen.

Sir HUMPHRY ROLLESTON (Cambridge) asked for further information about the data for the existence of two methods of circulation of blood through the spleen—one, perhaps, through the subcapsular area, the blood rapidly leaving the organ, the other of the storage nature, in which the blood remained there until it was wanted. He added that it would be interesting to know about the influence, inhibitory or otherwise, of the spleen on the bone marrow. How far had the spleen any special influence on immunity? Secondary as well as primary growths were very rare in the spleen; the rarity of secondary growths was perhaps partly due to the infection cells being drawn on into the liver; or was there some further power of resisting neoplasm in the spleen?

Dr. H. C. HOU (Pekin) reviewed the experimental work in progress in Pekin. The spleen was attached to the body wall and divided into halves. The upper portion had an intact nerve and blood supply while the lower half was denervated. A marked reduction in the size of both portions was observed after exercise, the diminution being more pronounced in the denervated segment. It was suggested that this was due to the liberation of adrenaline, as no change had been found in animals where both adrenal bodies had been removed. Spleens of doubly adrenalectomized dogs only contracted to a slight extent after death, while a marked reduction was found in the normal dog. Injections of adrenaline and pituitrin caused diminution in the size of the spleen.

Sir EDWARD SHARPEY-SCHAFER (Edinburgh) described experiments proving that the spleen was an organ most sensitive to adrenaline.

Sir BERKELEY MOYNIHAN (Leeds) remarked that recent investigations by the physiologists had borne out the anticipations of older writers. Pliny had stated that the spleen was enlarged in those who exhibited "immoderate laughter," and Maria in *Twelfth Night*, calling Sir Toby Belch when she was twitting Malvolio, refers to the same function. In *King John* the Bastard spoke of "spleen of speed to serve Your Majesty." Physiology had been termed a "laggard science." The surgeon ardently seeking for knowledge of many of the functions of the spleen turned almost in vain to the physiologists for answer to such questions as why all the splenic blood passed through the liver; why the veins from the tail of the pancreas carrying blood from the islands of Langerhans conveyed more blood than from the larger end of the gland; and why the blood from the large intestine joined the splenic vein before the blood from the small intestine. In connexion with splenic anaemia, many problems needed consideration and answer. The surgeon had shown, by his operations for haemolytic jaundice, one of the functions of the spleen. Sir Berkeley urged that physiologists should make their observations not only upon cats and dogs and mice, but upon men. Their work should be not only in the laboratory, but side by side with physicians and surgeons in the wards and in operating theatres. They would so give a purposeful direction to investigation and fill many gaps in the knowledge of functional anatomy.

Professor TAIT, in his reply, said that lymphoid tissue was not an essential element in the structure of the spleen. At the same time the circulatory and phagocytic arrangements present in the spleen showed a remarkable similarity to those adduced by Professor Herring as occurring in lymph glands. In the ellipsoids the blood first came into contact with reticulum, and foreign particles tended to adhere to the reticular fibres of these ellipsoids.

These particles were now collected and ingested by the sessile phagocytes of the ellipsoids, which thereafter migrated into the splenic parenchyma, just as the phagocytes of the lymphatic glands travelled when the empty ellipsoids underwent a regenerative process accompanied by hypertrophy. As Dr. E. S. Mills had shown, this process might lie at the root of splenomegaly. In answer to a criticism by Sir Almoth Wright, who objected to a division of the tissues into haemorrhagic and anaemorrhagic, on the ground that the blood coagulating principles were soluble rather than cellular, he stated that no tissue extract was active unless it contained cellular debris. He referred to recent work by Dr. Cleland and himself on the nervous machinery controlling rhythmic contraction.

THE KINETICS OF THE VARIOUS HAEMOLYTIC SYSTEMS.*

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THE object of an opening paper should admittedly be to arrange and to condense the subject so that it can be easily grasped even by those who are unfamiliar with it. In the case of the subject of haemolysis, with its many and diverse investigations, this is impossible; I propose, therefore, to fix at the very beginning the point upon which all these investigations must ultimately converge, and to deal with the subject by describing certain successive steps which lead up to the final problems; for the solution of these final problems, concerned as they are with the reactions occurring in complement-antibody systems, forms the principal justification for the labour spent on the study of simpler haemolytic phenomena. This treatment can only result in the presentation of the subject from a restricted point of view; it will, however, make the connexion between the simple and the complex forms of haemolysis as plain as is possible.

Before considering the action of even the simplest haemolysin it will be as well to be quite clear about the structure of the cell upon which it acts. All the recent investigations lead us to the conclusion that the red cell of mammals is a balloon-like structure possessed of an envelope of considerable strength. One of the clearest proofs of this is the fact that the changes of shape and size which the cell undergoes, both in hypotonic solutions and in solutions containing haemolysins, are those of a balloon-like body and not those of a sponge-like structure;¹ if more direct evidence is required, Seifriz² has actually microdissected the human red cell, stretched its envelope, and observed the escape of fluid containing haemoglobin. There is considerable evidence, from investigations on quite different lines, that the envelope is composed principally of protein and not of lipid, as was once thought; in this connexion may be mentioned the work of Oliver and Barnard³ on cell agglutination, of Seifriz on microdissection, as well as the results of the recent work on the haemolysins. The best conception of the structure of the envelope is probably one similar to Bechold's—a protein framework, in the interstices of which lipid is contained. Several proteins probably enter into the composition of the framework.

The action of the haemolysins must therefore be principally on this complex envelope, its continuity being destroyed, with the result that the fluid contents are liberated into the surrounding fluid.

1. Simple Haemolytic Systems.

Setting aside the instances of lysis by mechanical means, which tell us very little, the simplest cases of haemolysis are to be found in systems which contain one lysin of known constitution and in known quantity, acting on washed red cells, and acting in the absence of any substances which accelerate or inhibit the reaction. As an

example we may take the case of saponin acting on washed mammalian red cells at a constant temperature.

In such a case it is easy to satisfy oneself that the reaction which results in lysis is accompanied by a using up of saponin, and by a combination of the lysin with some component of the red cell envelope. This component is certainly protein in nature. The amount of saponin used up in producing lysis of any given number of cells can be very exactly measured, and the rate at which the using up proceeds gives us the rate of the reaction between the lysin and the protein component of the envelope with which it combines. This reaction we call the "fundamental" reaction. The factors upon which its velocity depends have been exactly determined, and it is known that the amount of saponin, x , used in producing lysis in time t , is given by the expression (1):

$$\kappa t = \log \frac{c}{c-x}$$

where c is the initial concentration of saponin present in the system, and where κ is a constant which varies with the surface presented by the cells, the temperature, and other factors. The fundamental reaction, which is described by this expression with great accuracy, is therefore a reaction of the first order, and its velocity at any moment dependent on the concentration of free lysin present at that moment.

Expression (1) thus gives the quantity of saponin combined with the cells at the end of any given time, but it does not, as it stands, give the amount of lysis which results from this combination. The reason for this is that the cells offer different resistances to the lysin, so that the least resistant cells haemolyse when only a small amount of their protein component is united with the saponin, whereas the more resistant cells require large quantities of the protein component to unite with the lysin before haemolysis takes place. On theoretical grounds we should expect that the cells, if grouped according to their resistance, would give a frequency distribution of a symmetrical type, the resistance of a few cells being small, the resistance of a few cells being great, and the resistance of the majority being intermediate. This is in fact the case, and the particular distribution, for human cells at least, is of the type shown in expression (2):

$$n = y_0 \left(1 - \frac{x}{a}\right)^m$$

Here n is the number of cells, expressed as a percentage of the whole for convenience, in any group of resistance x ; y_0 is the number of cells of mean resistance, and a and m are constants.

Now as the fundamental reaction proceeds according to expression (1), quantities of lysin x_1, x_2, x_3 , etc., combine with the cells in times t_1, t_2, t_3 , etc. The cells whose resistance corresponds to x_1 haemolyse when x_1 is formed, and, if we want to determine their number, we can do so by means of expression (2). When x_2 is formed, the cells whose resistance corresponds to x_2 haemolyse; this number is again given by expression (2). And so on for x_3 , and for all other values of x . The number of cells which are haemolysed at any moment from the beginning of the reaction until the stage of complete haemolysis is accordingly not to be found from any one expression, but from the simultaneous solution of expressions (1) and (2).

The result of this simultaneous solution is an S-shaped curve which gives the number of cells haemolysed at any stage of the experiment, and, as the fundamental reaction is steadily slowing down, the S-shaped curve is always asymmetrical. Recently several methods have been introduced for the study of such curves, by far the best being that which depends on the use of selenium as a means of measuring the intensity of the light transmitted by the cell suspension. By this method, which we owe to Mellanby, it can be shown that the S-shaped curves obtained from experiment agree exactly with those calculated from expressions of the form of expressions (1) and (2).⁴ The conclusion must be that the fundamental reaction between the protein of the envelope and the lysin is of the first order, and that the resistances of the cells is expressed by a frequency distribution of Pearson's Type II. That some explanation such as this is the probable one was suggested by Brooks⁵ in a theoretical

* A paper read in opening a discussion on haemolysis in the Section of Physiology and Biochemistry at the Annual Meeting of the British Medical Association, Edinburgh, 1927. Sir Edward Sharpey-Schafer, M.D., F.R.S., President of the Section, was in the chair.