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New trends in spleen research: Conclusion

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Tischendorf's scholarly paper on the evolution of the spleen complements his monograph on the spleen in von Möllendorf's Handbook and Jordan's chapter on Comparative Hematology in Downey's Handbook.

In nonmammals, Tischendorf indicates in his review of phylogeny, the spleen is irreplaceable as an hematopoietic organ. In man it becomes of only limited vital importance as a highly differentiated regulation center coordinated to the humoral system. Before the spleen separates to become an independent organ in selachii there is a lymphoid tissue in the midgut of cyclostomes that corresponds structurally and functionally to spleen and bone marrow of higher vertebrates. In nonmammalian vertebrates, Tischendorf quotes Murata that 'an elongated spleen extending the entire length of the gut is considered as the ancestral form and the variations of the shape and position are explained on the assumption that of the entire dorsal mesentery retains phylogenetically a development potentiality for forming splenic tissue, but an arrest of development either at the cordal or cranial portion takes place'. From birds to mammals the spleen lies in the dorsal mesogastrium as a tongue or bean shape organ with little variation in position. Tischendorf indicates that while the splenic capsule may contain variable amounts of muscle and connective tissue, a stout capsule and a system of trabeculae that forms chambers in the spleen is a relatively late phylogenetic development.

With regard to the splenic pulp, cyclostomes possess both lymphoid and myeloid components, but no separate white and red pulp, thereby resembling hematopoietic tissue in the gut submucosa. In selachian spleen arteries and veins have lymphoid sheaths, but again, white and red pulp cannot be sharply segregated.

White pulp and red pulp do develop in fish to varying degrees. The lobed spleen of dipnoi show a central lympho-mono- and thrombopoietic white pulp and a rind of sinusoidal erythropoietic red pulp, combining the three blood forming mammalian tissues. Despite well-developed periarterial lymphoid sheaths and follicles the separation of white and red pulp is much less distinct in reptiles than in mammals. Reptiles occupy an intermediate position in hematopoiesis between amphibians, where the spleen is the main site of lympho- and erythropoiesis, and birds where erythropoiesis is confined to bone marrow and lymph nodes appear as independent organs. It is in birds that white pulp reaches its highest state of evolution in nonmammals. Here secondary lymphatic nodules containing germinal centers first appear.

The destruction of erythrocytes in fish occurs in vascular beds, especially in lung and spleen. In other cold-blooded animals the destruction of erythrocytes may occur principally in the liver, as indicated by deposits of hemosiderin. In birds it takes place about equally in liver and spleen, and in mammals mainly in the spleen. Tischendorf touches on the nature of trabecular vessels, a theme taken up more fully by Faller in a subsequent paper. In his description of the vascular bed of the spleen, Tischendorf indicates that an interruption of a vascular wall within the spleen does not necessarily imply an interruption of blood flow. Even if one does not concede that all nonmammalian spleens have a structurally closed vascular bed all the evidence points to a functionally predominantly closed circulation in which there is a continuous direct transit of blood from the arterial to the venous vessels. This theme is taken up in many other papers in this collection, notably those of Fujita and his colleagues, the Hartwigs, and the McCuskeys. Tischendorf's discussion of the vascular beds of the spleen is valuable because of the perspective that a comparative treatment gives to an understanding of mammalian spleen. Tischendorf also discusses the extrasplenic vessels, a matter taken up also by Sinzinger and Firbas on the pathology of the splenic artery. As do others in this group, Tischendorf distinguishes those relatively large spleens rich in trabeculae and muscle but poor in lymphoid tissue as *storage spleens*, represented by the horse, but perhaps most completely by the elk. In contrast, smaller spleens poor in trabeculae and smooth muscle but rich in lymhpoid tissue may be regarded as *defense* or *metabolism* spleens. V. Herrath points out that storage spleens are reticulum spleens, defense spleens are sinusoidal spleens.

Finally Tischendorf addresses the question of innervation of the mammalian spleen, a matter taken up by other papers in this symposium notably those of v. Herrath and of Reilly.

Fänge and Nilsson deal rather fully with the fish spleen. They clearly review the development of the spleen from its midgut position in cyclostomes to its separated character in other fish. The close relationship of the spleen with the pancreas is emphasized. They present the architecture of the vasculature with emphasis on such structures as ellipsoids, well-developed in many fishes. While the splenic circulation in fishes is usually described as open, Fänge and Nilsson indicate that microcirculation studies have not been made. Lymphoid tissue is well developed in elasmobranches and holocephalans especially surrounding the arteries. But germinal centers are lacking, and periarterial lymphoid tissue is less distinct than in mammalian and avian spleens. Fänge and Nilsson report that antibody-producing cells have been found in teleost and other fish spleens and that the spleen appears to share immunologic capacity with the pronephric lymphatic tissue. The ellipsoid in fish may act as a valve, sphincter or filter. A major function of ellipsoids in fish is phagocytic, as in mammals. (I note, as discussed more fully later, Blue and I suggest the term *periarterial macrophage sheath* for ellipsoid.) Antigen-antibody complexes, moreover, may be taken up by macrophages in ellipsoids and transferred to melanomacrophage centers.

Melanomacrophage centers are nodules of pigment-containing cells, *melanomacrophages*, which occur in lymphohematopoietic tissues and/or the liver of most teleost fish. The melanomacrophages also contain cellular debris of host or parasitic origin. Dendritic cells containing immunoglobulins may also occur in these centers. The authors indicate the structure and function of the ellipsoids, the melanomacrophage centers, and the relationships between these and the lymphoid tissue of fish spleen are insufficiently known and ought to be further investigated. Perhaps melanomacrophages centers are antibody producing structures, in some respects similar to the germinal centers of birds and mammals. Fish spleen may store blood. In the Rhine salmon almost a quarter of the total blood volume may be stored, to be ejected during asphixia or the administration of certain drugs.

With regard to hematopoiesis, erythrocytes and thrombocytes are produced in elasmobranch spleen and granulopoiesis occurs in the spleen of the electric ray. Erythropoiesis is intravascular as it is in birds. In teleosts hematopoiesis occurs predominantly in the pronephros or metanephros but in some species the spleen is active. The fish spleen is a main site for the destruction of red blood cells although the details are not well known. The high content of iron within the melanomacrophages may be a consequence of red cell destruction. The fish spleen is innervated by sympathetic splanchnic nerves, with no indication of vagal innervation. It is believed that one consequence of the splanchnic nervous control of fish spleen is to induce erythrocyte release from splenic stores during stress, as hypoxia. The fish spleen contracts in response to adrenalin and noradrenalin.

The contribution of Hartwig and Hartwig emphasizes the evolution of the capsule and trabeculae of the spleen, relating these structures with storage and rapid release of erythrocytes. The Hartwigs provide a valuable review of the work on the spleen's capacity to store and express blood. Von Herrath introduced the concept of two extreme types of spleen, the *storage* type and the *defense* type with a graded series between (see also Tischendorf's paper in this issue). Spleens that store and release considerable numbers of red cells as the equine spleen are characterized by high relative weight, many trabeculae rich in smooth muscle and a small volume of white pulp. The spleen of rabbits on the other hand, have low relative weight, few trabeculae containing smooth muscle cells and relatively rich lymphatic tissues. The former spleen is the storage type, the latter the defense type. Von Herrath correlated the presence of smooth muscle in trabeculae and the effectiveness of red cell release. The spleen of the elk appears to have the highest capacity to store and release erythrocytes. The Hartwigs accept von Herrath's classification but have critically reviewed the criteria by which he has scaled and defined spleens. They find, for example, stress during the sacrificing procedure may rapidly decrease the spleen's volume. Domestication of animals carries with it certain selective breeding, moreover, that makes comparison with wild animals difficult. Selection of morphometric procedures may also affect results. The Hartwigs have developed a staging procedure evaluating the occurrence and distribution of smooth muscle cells and the existence of a mono- or bilayered capsule. They recognize four types of spleen; 1) a monolayered capsule and trabeculae composed mainly of connective tissue, 2) a monolayered capsule and trabeculae rich in smooth muscle, 3) a bilayered capsule and trabeculae rich in smooth muscle, and 4) a bilayered capsule and trabeculae rich in smooth muscle accompanied by additional smooth muscle cells in the red pulp. A bilayered capsule contains, in its subserosal portion, rich networks of veins and lymphatic vessels which appear to transport the fluid compartment of the blood separated from erythrocytes allowing efficient storage of large volumes of high hematocrit blood. The Hartwigs go on to compare the functional capacities of the spleen of different species bringing in considerations of phylogenetic position in evolution, ecology and such behavioral features as diet and ecology. It appears that only mammals evolving late possess spleens with a bilayered capsule and trabeculae rich in smooth muscle cells. That the type 4 spleen which contains muscle cells in the pulp should possess the highest capacity to store and release red cells is supported by the observation that this type of spleen receives the highest density of sympathetic innervation revealed by cytochemistry of catecholamines. This classification correlates well with the observations of Blue and Weiss and of Tablin and Weiss in the dog and horse respectively, that reticular cells in the red pulp contain many microfilaments and are innervated by adrenergic nerves. The Hartwigs indicate that contractile filaments have been localized in a number of different reticular cells using immunocytochemical techniques.

Faller contributes an important study on the connective tissue structure of the human spleen dealing in depth with a relatively neglected subject, the splenic capsule and trabeculae. He emphasizes the value of the advanced fetus in studying patterns of structure. The capsule itself is coarse 1.1–1.5 mm thick, covered except at the hilus by a serosa. The capsule contains two layers of white connective tissue distinguished by the direction in which their fibers run. While most collagen fibers are of uniform, medium thickness they become considerably finer in the transition zone toward the pulp and may even pass directly into the reticulum. Fine elastic fibers form a system in the superficial connective tissue layer. In the deep layer elastic tissue is coarser. Faller distinguishes different types of trabeculae; vascular, venous, connecting, fixation, and radial. The vascular trabeculae form the stem of the trabecular system, the major branches containing both arteries and veins. The branches of these trabeculae form *arbor trabecularis* limiting the inner part of the splenic center. The lobules they form are relatively large. Trabecular veins follow the branches of the trabeculae for a much longer distance than trabecular arteries. Connecting trabeculae containing no vessels branch laterally from the terminal branches of the arbor trabecularis. The connecting trabeculae serve to strengthen the arbor trabecularis and to limit the splenic lobule. They also form the inner limit of the subcapsular zone, the zone lying between them and the inner surface of the splenic capsule. Indeed, from the inner side of the splenic capsule radial or spoke trabeculae grow in the direction of the trabecular

beam, the structure formed by the lateral trabeculae in aggregate, forming with the trabecular beam, the tangential beam net. The subcapsular zone thus forms an envelope or mantle, surrounding the splenic center. The subcapsular zone contains no white pulp. Faller reports that reticular fibers on micromanipulation have considerable tensile strength but do not stretch. By folding and unfolding, however, they can provide for considerable change in splenic volume. By careful delineation of capsule and trabeculae Faller defines the splenic lobules and their vasculature and provides important implications in rationalizing the volume control and elasticity of the spleen.

Fujita and his colleagues have contributed a valuable paper on scanning electron microscopy of the circulation in the human spleen. Their techniques, refined over many years, include an adaption of the conductive staining method of Muracomi which, through long-term immersion in tannic acid and osmium tetroxide, provides the tissue electrical conductivity as it is heavily impregnated with osmiun. This avoids charging which would otherwise diminish image quality. Little is said of the white pulp but note is made of perforations in the circumferential reticulum allowing passage of lymphocytes from white pulp to marginal zone. The rich vascular supply of the marginal zone derived from follicular arteries and from recurrent penicillii is also noted. In their study of the endothelium of the splenic sinus Fujita and his colleagues emphasize the presence of side bars consisting of lateral processes of the rod cell endothelium connected to one another forming bridges and thereby a lattice pattern. They indicate correctly, moreover, that Chen and I were in error when we wrote that in the human sinus the wall consisted of simple rods arranged side by side without junctional complexes. A number of authors, including my colleagues and me, subsequently have demonstrated junctional complexes between rod cells. In the scanning microscope the junctional complex traverses the side bridge of rod cells, showing a suture-like surface appearance. Fujita and his colleagues claim that the gaps or perforations in the sinus wall are persistent openings, which provide routes for passage of the cellular elements of the blood through the sinus wall. I continue to take the position that while blood cells and other elements certainly pass between the rod shaped endothelial cells of the wall of the sinus and in so doing create gaps, when the intercellular slits between endothelial cells is not occupied by cells, or widely distended by fluid, the endothelial cells lie smoothly side by side without any gaps. In my experience a frequent artifact of scanning electron microscopy (which requires drying of blocks of tissue) is the separation of the lateral margin of the rod shaped cells from one another, they being maintained in contact only by the membrane junctions. The results is not unlike the so-called 'intercellular bridges' observed by light microscopy between endothelial cells of the uterine cervix, for example, where due to fixation the epithelial cells are retracted from one another contact being maintained only at the junctional complexes. In my opinion, the best evidence that most of the endothelial cell gaps in splenic sinuses represent an artifact of scanning electron microscopy is that these gaps are seldom present in well-fixed tissue studied by transmission electron microscopy. Instead the rod-shaped endothelial cells lie smoothly and closely side by side. Fujita and his colleagues go on to carefully and usefully define the basal surface of the sinus endothelium and the nature of the splenic cords, including a characterization of macrophages (whose surface is beset with filopodia) and reticular cells (whose surface is smooth). Careful delineation of the termination of arteries indicates that the circulation is anatomically open. Three types of terminal arteriole, with gradations between, are recognized. In the first, the endothelium of the arterial end portion becomes perforated or split and fans out in funnel shape in the cordal spaces. In the second, the end portion is sacculated forming an ampulla, which may be perforated by small rounds pores. The third type is formed by a connection of two or more penicillar ends into a larger ampulla or cave. Complex caves represent a newly discovered arterial terminal character, and represent, in human spleen, the remnants of the so-called marginal sinuses known in the rat and the mouse. The authors go on to a useful and extended critical discussion and review of the nature of the arterial terminals, defending the existence of an open circulation. I concur with their conclusions. Interestingly, among the points they make is that the slit in an arterial termination may rarely overlap a sinusal perforation without intervention of a recognizable amout of cordal space (see their figure 9, reference number 18). In the short paper I have contributed with Powell and Schiffman, a transmission electron micrograph (figure 7) appears to corroborate Fujita's scanning electron micrograph.

Reilly's work, an important anatomic and pharmacologic study of the innervation and vascular pharmodynamics of the spleen, considers the effects of vasoactive substances and sympathetic agonists and antagonists and other inhibitors on the capsule and vasculature of the mammalian spleen. The innervation of the spleen is sparse. Adrenergic or sympathetic nerves have been demonstrated in many spleens using histochemical and electron microscopic techniques. There is limited cholinergic or parasympathetic or sensory innervation. Adrenergic nerves supply arterial smooth muscle of human and mouse spleen, while in the rat, cat and dog they also innervate the smooth muscle of capsule and trabeculae. I suspect that in storage, in contrast to defense spleens, the innervation to capsule, trabeculae and perhaps to even the smooth muscle or reticular cells of the pulp is well developed. Indeed, Reilly and his coworkers found reticular cells of murine white pulp, Blue and Weiss reticular cells of the canine red pulp, and Tablin and Weiss reticular cells in equine red pulp, innervated by adrenergic nerves. These reticular cells, which must be contractile because of their high concentration of microfilaments, probably participate, along with the smooth muscle of capsule and trabeculae, in causing a large decrease in splenic volume following adrenergic stimulation.

Reilly goes on to discuss, in valuable detail, vascular pharmacodynamics (including adrenergic mechanisms, cholinergic mechanisms, vasoactive amines, nucleotides, lactic acid, prostaglandins, prostacyclin-thromboxane and polypeptides) indicating the extraordinary effects,

including nuances and paradoxical effects, obtainable – and the variations dependent upon species and physiological condition. His last section is on unresolved problems. Here Reilly critically reviews certain of these results indicating that caution must be applied in the interpretation of pharmacodynamic and neurophysiologic data on blood flow. For example, drugs in the blood stream or affixed to vascular receptors peripheral to the spleen may have an effect on splenic results. Technique and dosage may cause different and apparently conflicting results. Such cells as macrophages produce prostaglandins and others cells may release these or other substances that influence intrasplenic blood flow. Differences in splenic structure, moreover, as the difference between sinusal and nonsinusal spleens, or, as discussed in a number of papers in this multi-authored review, differences between storage and defense spleens may underlie differences in pharmacological results. Further, the splenic microvasculature is difficult to get at. Most information has been obtained from innervated vessels larger than 300 μm and not the microvasculature. The sites of chemical interaction within the resistance vessels of the spleen, the role of hormones and the role of other stimuli on this microvasculature have not yet been studied.

Seifert and Marks' paper on the regulation of hemopoiesis in the spleen nicely complements those of the Dutch investigators that follow it. They begin with a review of the ontogeny and phylogeny of hematopoiesis covering from a different angle some of the work discussed more fully by Tischendorf, Fänge and Nilsson and the Hartwigs. Seifert and Marks discuss the important immunolgic role of the spleen in reptiles and mammals and its less important role in fish and amphibia, where the spleen may possess more general hematopoietic functions. They review the rearrangements in heamatopoiesis imposed by bone marrow, a later arrival, but a tissue which has a compelling preemptive relationship with hematopoiesis. Seifert and Marks discuss the population of hematopoietic stem cells that the spleen acquires making the important point that a permanent excess of pluripotent stem cells relative to committed stem cells is retained in murine spleen, providing hematopoietic activity throughout life. The bone marrow, however, contains many more pluripotent stem cells and prevails as the dominant hematopoietic organ. Splenic hematopoiesis may be less significant in rats than in mice. But even in mice splenic erythropoiesis, a type of hematopoiesis Seifert and Marks do not consider, is minor relative to marrow in normal individuals. After reviewing the well-known interactions of spleen and bone marrow Seifert and Marks raise the question of the nature, if any, of the communications between bone marrow and spleen and whether the factors that regulate bone marrow are the same as those that work on the hematopoietic cells of the spleen. I believe that while they have not been defined, it appears that there are humoral connections between bone marrow and spleen, as shown, for example, by Wyler and his colleagues, in methylcellulose-loaded or malarial spleens. Seifert and Marks advance a number of interesting model systems in exploring hematopoietic functions in the spleen. They deal, for example, with an absence of bone marrow space as occurs in osteoporosis, a phenomenon to which these investigators have given considerable study. Here, the spleen and not the liver, shows a considerable amount of extramedullary hematopoiesis. The authors go on to discuss splenic hematopoiesis during marrow dysplasia and rather fully consider myeloproliferative disorders indicating that these represent a group of diverse poorly understood blood diseases that include chronic and acute myeloid leukemia, polycythemia vera, essential thrombocytopenia and agnogenic myeloid metoplasia. These diseases appear to have in common a clonal basis and have been termed clonal hemopathies. As a further example of splenic hematopoiesis secondary to bone marrow failure the authors consider the bone marrow depletion following the administration of strontium 89.

Using a variety of polyclonal and monoclonal antibodies as reagents in immunocytochemistry van Ewijk, van Vliet, and Nieuwenhuis determined the localization of subgroups of T and B cells in the white pulp. They also determined migration pathways and kinetics within white pulp, of T and B lymphocytes obtained by thoracic duct cannulation and radiolabeled. Van Ewijk and his colleagues show the white pulp as an organized lymphoid compartment demarcated from marginal zone and red pulp. T and B cell domains were defined. T cells may reside in B cell zones and B cells may lie at the border of T cell domains. But only a subpopulation of T cells, T helper cells, are found in B cells areas. Thus only those T cells needed to trigger B cells are present among the B cells. Van Ewijk and his colleagues found that T and B cells entered the spleen by the same route, the marginal zone, and for a time both B cells and T cells travelled along the same route, moving through marginal zone, along terminal arterioles to the periphery of the periarterial lymphatic sheath. Here T cells separated from B cells by penetrating further into the periarterial lymphatic sheath, while B cells, skirting the T cell zone, continued toward the base of the follicles and eventually segregated themselves from T cells by entering the corona of lymphocytes surrounding the lymphatic follicle. Now the marginal zone in rodent spleen receives a high concentration of arterial vessels and is, thereby, a major site for first receiving whatever comes into the spleen. Large-scale antigen-trapping occurs here, as shown by this group of investigators. The marginal zone contains metallophil cells and specialized macrophages, which may well be closely related. These authors indicate that a monoclonal antibody selectively staining them has been developed in their laboratory. Of the questions arising from this valuable work and from the important work of Eikelenboom and his colleagues in the next paper is the relationship between the metallophil cells, interdigitating cells and other stromal cells that have an accessory role to lymphocytes in antigen presentation and other phenomena, as migration. van Ewijk and his colleagues address the matter of the plasmacellular reaction or the differentiation of B cells to plasma cells in an antibody response. They found that in a progressive immune response plasmablasts accumulate in the periarterial lymphatic sheath and leave white pulp travelling along the thin reticular sheaths surrounding terminal arterioles. Finally plasma cells

occur in red pulp close to trabeculae and sinuses. van Ewijk speculates that recirculating B cells are antigen-reactive and enter T cell domains in order to obtain the stimuli they need to differentiate into antibody forming cells, thereby accounting for the presence of Ig-containing plasmablasts in T cell domains. The authors indicate that at present there is no clue to the mechanisms of homing of lymphoid populations into their respective domains. Cell surface determinants of non lymphoid cells may play a part. Lymphocytes recirculating through lymph nodes, van Ewijk and his colleagues remind us, have a receptor for specific determinants on the luminal surface of endothelial cells in high endothelial venules. They and others have postulated the possibility of similar molecules on stromal cells in white pulp which may be reponsible for migration and homing.

Increasing attention is being paid to the stromal or non lymphoid cells in hematopoietic tissues in relationship to control of migration, homing differentiation and establishment of the microenvironment. Using immunocytochemistry dependent upon the specific linkage provided by monoclonal antibodies to a variety of cell types Eikelenboom, Dijkstra, Boorsma and van Rooijen effectively characterize lymphoid and non lymphoid cells in a histological context. They present the morphology of white pulp indicating among other things, the distribution of reticular fibres and cells. They confirm, again, that T cells are present mainly in the central area of the periarterial lymphatic sheath and B cells in the peripheral part of the sheath, in follicles, and in the marginal zone. They find, moreover, as have others, that marginal zone B cells are different in type than follicular B cells. Marginal zone lymphocytes are medium-sized and have strong IgM membrane staining, while follicular lymphocytes are small and show only moderate IgM staining. The authors usefully characterize nonlymphoid cells. They indicate that reticular cells are fibroblastic cells which synthesize reticular fibers. I believe the evidence for such synthesis is persuasive, but circumstantial. Follicular dendritic cells occur in follicles while interdigitating cells lie in the central area of the periarterial lymphatic sheath. Both cell types are capable of holding antigen-antibody complexes at their cell surface. Another cell type similar to reticular cells, follicular dendritic cells, and interdigitating cells in having a dendritic morphology can be found among adherent cells of cell suspensions prepared from murine peripheral lymphatic organs. These cells are called dendritic lymphoid cells and are confined to the white pulp of the spleen. At the periphery of white pulp there is another nonlymphoid cell the so-called marginal metallophil. Eikelenboom and his colleagues go on to characterize each of these cells and to review information about them.

In characterizing stromal cells, I believe we are clearly dealing with a matter of the greatest importance in terms of such phenomema as antigen presentation, control of migration and homing, creation of microenvironments, stimulation of differentiation and hematopoiesis. The interrelationships of these cells must be explored. Are they variants of the same cell? Are they different in source and development? To some extent stromal cell biology has its parallel with hematopoietic cell interrelationships. It can be recalled that for many years the monophyletic-polyphyletic dichotomy polarized studies in hematopoiesis, one group of scholars believing that lymphocytes are multipotent, identical or simply variants of monocytes, hemocytoblasts and some other cells while other scholars believed that these cells were different from one another. The relationship of stromal cells to one another remain to be explored. Are Langerhans cells, interdigitating cells, metallophil cells, veiled cells, lymphodendritic cells, etc., the same cell type or are they different? Have we one class of cells which serves T cells and another class that serves B cells? The interrelationships of hematopoietic cells and the monophyletic vs polyphyletic schism have been elucidated. Experimentally a part of the solution was, as an investigator whose name eludes me has nicely put it, the ability to mark cells indubitably, innocuously and indelibly and tracing their course in histological context. Eikelenboom and his colleagues have very nicely set the stage for such further work. They conclude their paper with a discussion of plasma cell differentiation that complements that of van Ewijk and his coauthors. The collection concludes with three papers on the pathology of the spleen. In his splendid review on the role of spleen in leukemia and lymphoma Maurer points out that the spleen may be the site of the first or only clinical manifestation of neoplasia and therefore the evaluation of the spleen may be essential in establishing the disease. But in the leukemias, although splenomegaly may be the leading sign and cause trouble, as thrombocytopenia, displacement of other organs, and spontaneous rupture, splenic involvement is more in the sense of a bystander reaction. The leukemias, particularly chronic myelogenous leukemia, are the most ostensible examples involving red pulp. Pulp cords and sinuses are infiltrated and only secondary is the white pulp obliterated. Hairy-cell leukemia is characterized by splenomegaly and cytopenia and is the only disease causing diffuse red pulp involvement where macroscopically the red pulp is fleshy without nodules. Prominent pseudosinuses constitute a unique feature of hairy-cell leukemia, the sinuses being pools of red cells or hairy-cells lining a lumen, sitting on attenuated endothelial cells.

As regards malignant lymphomas on the non-Hodgkin's type presentation in the spleen is rare. Involvement of the spleen in the course of non-Hodgkin's lymphoma presenting in other sites is more frequent, occurring perhaps in 35 to 40% of cases. Lymphomas typically affect white pulp causing nodules. Staging laparotomy is less important than in Hodgkin's disease since a greater proportion of cases presents with generalized disease making the recognition of splenic involvement less likely to alter treatment. But for patients with diffuse histiocytic non-Hodgkin's lymphoma in stage 1 the demonstration or exclusion of splenic involvement is crucial. Hodgkin's disease is a neoplastic process which begins confined to one site and typically spreads slowly. The spread is by continuity, following an orderly predictable path from one lymph node chain to another to which direct lymphatic channel communication exists. Splenic involvement is the basis for spread to liver and bone marrow.

The presence or absence of Hodgkin's diesease in the spleen is therefore important and the reason for staging laparotomy and splenectomy. The spleen is involved in a nodular fashion, the earliest focal lesions occurring in marginal zone or follicles. Because a staging laparotomy may contain only a solitary tumor of one to a few millimeters in diameter, Maurer emphasizes that the spleen must be bread-loafed to slices less than 5 mm thicknesses. Histological diagnosis of Hodgkin's disease in any location depends upon the demonstration of Reed-Sternberg cells in an appropriate cellular background. Maurer goes on to discuss the impairment of cell mediated immunity in Hodgkin's disease before turning to malignant histiocytosis, as the last of the neoplasias considered. This is a systemic neoplasia of histiocytes with splenomegaly as the leading sign. The malignant histiocytes difusely infiltrate red pulp, and may encroach upon white pulp. Nuclear pleomorphism and erythrophagocytosis characterize the disease. Massive spenomegaly may occur as the only sign. Where the histiocytic and the phagocytic capacities are not evident immunocytochemical reaction for muramidase and chymotrypsin may be useful in defining the histiocytic nature of tumor cells. Differential diagnosis must consider hairy cell leukemia and diffuse histiocytic non-Hodgkin's lymphoma as well as Hodgkin's disease.

Sinzinger and Firbas present a most comprehensive and thoughtful study of splenic artery disease and disclose profound arteriosclerotic changes that occur in this vessel, having analyzed about 1500 human splenic arteries over a period of 10 years. The arteriosclerotic regions include fat infiltration, fibrous plaques, and calcification. The authors consider of the intima-media index, studies of the internal elastic membrane, discussion of smooth muscle cells, of parietal thrombus formation, of aging changes, and of the regulation of hemostasis. The authors demonstrate that at very early ages the human splenic artery is subject to severe arterosclerotic alterations. The reasons this artery undergoes such profound alterations and the clinical implications are not yet known.

Please turn to the summarized papers and to that of Weiss, Powell and Schiffman for references.

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Short Communications

Novel rearrangement of purines

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Summary. 6-Trichloromethyl-9-methylpurine (**1**) rearranges to 6-dichloromethyl-9-methyl-8-oxopurine (**2**) in aqueous mild acidic solution. The rearrangement is rationalized in terms of a reaction involving protonation, covalent hydration, prototropic equilibrium and/or a hydride transfer. An alternative mechanism involving a 'positive' halogen compound and hypochlorous acid as an intermediary is also proposed. Compound **1** condenses with 4,5-diaminopyrimidine to give the purine-pyrimidine Schiff base pair **4**.

Key words. Purine rearrangement; synthetic purine-pyramidine pairs; 'positive' halogen compound; 6-trichloromethyl-9-methyl purine; 6-dichloromethyl-9-methyl-8-oxopurine; REDOX reaction.

'The essential feature is the base pairing'[2]. Since the elucidation of the structure of double-stranded DNA this phenomenon has continued to serve as a focus of activity both theoretically and experimentally[3]. Synthetic purine-pyrimidine pairs, particularly dinucleotide analogues of the type B-C_n-B', have been explored by Leonard as convenient models for studying interactions of nucleic acids bases in the absence of various complicating factors[4]. We have directed our efforts towards synthetic purine-pyrimidine pairs in which the bases are covalently connected by an amide or an imine linkage[5], as a lead to the development of anti-cancer agents[6].

Base pairs of the type Pu-CONH-Py were first prepared by Cohen and coworkers by condensations of 6-trichloromethylpurine with aminopyrimidines, and reportedly displayed unusual spectroscopic (UV and fluorescence) features[7-9]. In our attempts to synthesize Pu-CONH-Py pairs with fixed purine tautomers, we have investigated the behavior of N-methyl-6-trichloromethylpurines towards nucleophiles. We report a novel rearrangement of 6-trichloromethyl-9-methylpurine (**1**) into 6-dichloromethyl-9-methyl-8-oxopurine (**2**) in aqueous solution under mild acidic conditions. In the presence of aminopyrimidines, the reaction led to the formation of purine-pyrimidine Schiff base pairs.

6,9-Dimethylpurine[10-12] was chlorinated with sulfuryl chloride in trifluoroacetic acid at 60 °C to give **1** as colorless crystals, m.p. 146–148 °C in 57% yield[7]. ^{1}H NMR[13] δ ($CDCl_3$) 3.98 (s, N-CH_3), 8.24 (s, H-2), 9.06 (s, H-8). δ (Me_2SO-d_6) 4.03 (s, N-CH_3), 8.90 (s, H-2), 9.19 (s, H-8). ^{13}C NMR[13] δ(Me_2SO-d_6) 30.88 (q, J = 132.4 Hz, N-CH_3), 95.37 (s, CCl_3), 128.48 (s, C-5), 149.45 (d, J = 214.8 Hz, C-8), 150.95 (d, J = 192.2 Hz, C-2), 154.47 (s, C-6), 155.30 (s, C-4). δ(H-8) and δ(C-8) are shifted downfield by 0.6 and 2.6 ppm in **1** relative to 6,9-dimethylpurine[12] indicating a considerable localization of a positive charge at C-8. The NMR evidence does not indicate such an effect at C-2, the corresponding shifts of δ(H-2) and δ(C-2) being 0 and −1.4 ppm, respectively.

Heating an aqueous hydrochloric acid solution (5%) of **1** at 100 °C under reflux for 24 h afforded **2** as colorless crystals, m.p. 189–191 °C (dec) (C_6H_6 or Me_2CO) in quantitative yield. Mass spectrum m/e 234 (23%), 232 (36.5, M^+), 199 (50.3), 198 (15.4), 197 (100, M-Cl), 168 (5.7), 161 (6.7), 156 (18.2), 143 (5.9), 140 (6.6), 134 (31.9), 115 (13), 113 (37.7), 86 (20.9). ^{1}H