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Relation of splenic functions to hematopoietic recovery following radiophosphorus intoxication in the Albino rat

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THE RELATION OF SPLENIC FUNCTIONS TO HEMATOPOIETIC RECOVERY FOLLOWING RADIOPHOSPHORUS INTOXICATION IN THE ALBINO RAT

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INTRODUCTION

Within the past four years Jacobson and associates of the Argonne National Laboratories, Chicago, have been concerned with several studies which were designed to demonstrate the role of the spleen in recovery from radiation injury. One of their earliest reports (Jacobson and Simmons, '48) describes the interesting observation that ectopic blood formation in the spleens of mice injected with a dose of 2.0 microcuries radiostrontium per gram of body weight is sufficient to obviate the development of anemia even though the bone marrow is largely destroyed and only gradually reconstituted over a period in excess of 100 days. However, splenectomized mice given this dose develop a severe anemia, recovery from which occurs only as the hematopoietic activity of the marrow recovers.

In pursuing this problem this group of investigators subsequently turned to a more positive approach. They found that subjecting mice to 600 r total body X-radiation would produce a severe anemia, leucopenia and thrombocytopenia (Jacobson, Marks, Gaston, Robson and Zirkle, '49). However, if the spleens were exteriorized and lead-protected during total body irradiation with 600 r ectopic erythropoiesis compensated with such rapidity and so extensively for the destruction and interruption of activity in the marrow spaces that no anemia of significance would become apparent. Ectopic granulocytopoiesis and megakaryocytopoiesis in the lead-

protected spleens compensated significantly but at a slower pace and less completely for the bone marrow destruction. These authors found that survival roughly parallels hematopoietic recovery up to 1,025 r total body radiation but that lead-protection of the spleen more than doubles the LD-50 dose of total body irradiation for mice which they estimated to be circa 550 r.

This phenomenon is not specific for the mouse. Shielding of the spleen of rats, guinea pigs and rabbits is followed by more rapid recovery of hematopoietic tissue (Jacobson, Simmons, Marks, Robson, Bethard and Gaston, '50). Furthermore this phenomenon is not specific for the spleen. Lead shielding of the appendix in rabbits will also hasten recovery (Jacobson, Simmons, Marks, Robson, Bethard and Gaston, '50; Jacobson, Robson and Marks, '50). However, these appendices show no ectopic blood formation up to 1000 r. Apparently the development of major sites of ectopic blood formation is not essential and probably not the deciding factor in the recovery of hematopoietic tissue or indeed the survival of the animal.

In previous work carried on in the Department of Anatomy of the University of Nebraska College of Medicine studies were made of the hematological effects of radioactive phosphorus intoxication in albino rats (Latta and Rosenlof, '49; Latta and Vose, '50; Latta and Kamprath, '51). Observations of ectopic erythropoiesis, granulopoiesis and megakaryocyto-

poiesis in the spleen were consistently made at dosages of 1.0 microcurie radiophosphorus per gram of body weight. This phenomenon would be apparent by 48 hours after treatment and could be found to be present over a period in excess of 48 days. These animals showed a leucopenia but no anemia and although the bone marrows were only slightly hypoplastic the granuloblasts suffered at the expense of the erythroblasts thereby producing relatively erythroblastic marrows. It was concluded from these findings that the marrow was prevented from acting maximally because of the continuous insalt being delivered to it from the radioactive phosphorus depositions in the bone and that ectopic myelopoiesis in the spleen was necessary to maintain an adequate blood picture. It was reasoned therefore, that after removal of the spleen radioactive phosphorus treatment should result in a leucopenia of a more severe nature and possibly an anemia. To determine the soundness of this reasoning the project being reported in this paper was undertaken.

Any attempt to understand what role the spleen plays in compensation and recovery from radiation injury must be superceded by an effort to understand splenic functions. The following is a brief historical review of the facts concerning splenic functions as they are understood today.

HISTORICAL

It has been known since ancient time that the spleen could be removed without fatal consequences. Therefore this negative method of approach has always been a favorite one. It is often repeated that the ancients removed the spleens of runners to make them more speedy and the practice is thought to have been due to the erroneous belief that the speedy giraffe had no spleen. Support of this theory has unexpectedly been produced in recent times from a reliable source. Macht and Finesilver, '22, found that the muscular integration of splenectomized rats was improved and that over a known length of cotton rope, their "running speed was certainly shortened," but offered no explanation for the improvement found. The possibility must not be overlooked that enlarged pathological spleens undoubtedly were common in ancient time, and the thread of truth in the ancient belief was perhaps due to the fact that runners probably would be speedier if relieved of a big chronic malarial or syphilitic organ.

There is little to detain us in the various conjectures about the spleen before the middle of the 19th century. Its swelling in certain acute infections was apparently recognized by the Greeks, but the ubiquity of malaria, even in classical times, here clouds the issue and "lien magnus" was quite impartially ascribed as the cause of bad breath, spongy gums, indolent ulcers, and so forth. A voracious

appetite after removal of splenic function, an idea which still crops out occasionally in the literature, seems to have been first observed, together with polyuria, by Malpighi. These are Malpighi's words: "In a dog of yet tender years a wound was made in the left hypochondrium, and the blood vessels of the protruding spleen and attached omentum were ligatured with a thread close to the hilus of the spleen; everything was presently replaced in its former position, the peritoneum and the muscles were sutured and the skin loosely united. After a lapse of a few days, the wound was healed. After some weeks the animal was strong enough to perform with enjoyment all its proper functions; so long as it lived no trace of any interference with health could be observed. Having become more hungry than before, it took its meals eagerly, devouring bone and foods of all kinds. One thing only I observed, namely, that it made water abundantly and most frequently, in fact continually. Though all dogs are continually doing this, it seemed in this respect to outdo all its fellows. Its habit of body was in every respect sound; indeed it became fat, and in other respects, in quickness and alacrity it equalled it fellows." (Krumbhaar, '26).

Toward the end of the 19th century with the improvement in histological technique, it had become established (1) that the spleen was not necessary for life and that after

removal its functions were apparently taken over by other organs, such as the bone marrow; (2) that the white pulp surrounding the small arterioles (lymphatic sheaths and Malpighian follicles) was concerned with formation of some of the colorless corpuscles of the blood (Virchow, 1846); and (3), that it could select various particles from the blood stream, including erythrocytes (Ponfick, 1885) and therefore was concerned with blood destruction.

Before continuing on to the review of splenic functions as they are understood today it should be mentioned that in comparing the results of investigators on splenic functions the size or weight of the spleen relative to the body weight of individuals of various species must be taken into consideration even when attention is confined to mammals. As the spleen in many of its functions forms perhaps the most important part of the reticulo-endothelial system such variations may be balanced by equivalent changes in other parts of the system. It should hardly be necessary to point out that many of the apparent contradictory results found after splenectomy by investigators using different species of experimental animals are probably to be explained partly on these grounds. Another important item in the evaluation of splenic functions is the age of the individual under examination. It is most active in the young and normally becomes smaller and more fibrotic with age.

ANATOMIC CONSIDERATIONS: The spleen consists of a richly vascular cavernous tissue surrounded by a capsule and subdivided by radiating trabeculae which contain vessels, nerves and scattered smooth muscle fibers. Scattered through the pulp are small pin-head sized grayish white nodules, the malpighian corpuscles (white pulp). These are masses of lymphoid cells that lie in intimate relation to the central arterioles. The red pulp consists of an intricate network of reticular cells and fibrillar reticulum with a rich mesh of blood sinuses formed by these elements.

The cellular elements of the spleen consist of fixed reticular cells, free reticular cells (macrophages), endothelial cells lining the sinuses, all the blood elements present in the circulation, small lymphocytes primarily in the malpighian corpuscles and free in the meshes of the red pulp stroma, lymphoblasts and large lymphocytes mainly in the germinal centers, occasional plasma cells, and erythroblastic cells and granuloblastic cells in the rat (Perla and Marmorston, '35).

The spleen presents four rather obvious anatomic or histologic subdivisions, upon which its functioning is dependent and from which pathologic manifestations may be expected to arise: (a) The smooth muscle capsule and trabeculae which make this organ physiologically contractile and expansile; (b) the vascular system with fenestrated sinusoids and/ or a

vast network of dilated capillaries ideally placed and spaced for the temporary sequestration of cellular reserves from the circulating blood; (c) the lymphoid system, identical in every respect with lymphopoietic tissue elsewhere in the body; and (d) the reticulo-endothelial system, comprising physiologic phagocytes morphologically similar to, but frequently uniquely dissimilar, at least in quantitative phagocytic function, from the reticulo-endothelial cells distributed elsewhere in the body.

It is a biologic axiom that the structure of any given tissue or organ is directly related to, and responsible for, its physiologic functions; and the pathologic corollary to this axiom is, that, given any known normal organic function, one may expect to find, sooner or later, a corresponding hypodysfunction or hyperdysfunction.

The spleen and its muscular capsule: Since the early experimental studies of Barcroft, '25, the physiologic reservoir function of the spleen for red blood cells has been repeatedly confirmed and generally accepted. Lauda, '33, after extensive review of the leterature and from his own observations, held that the "depot function" of the spleen for red blood cells in the human is of much greater importance in pathologic than in normal states. He concluded, however, that the techniques of comparative splenic artery and vein studies at that time were not sufficiently accurate to supply

significant proof. Watson and Paine, '42, made comparative splenic arterial and venous counts of the spleen at the time of splenectomy in selected human subjects. They found, after epinephrine, marked increases in erythrocyte concentration in the splenic venous blood, associated with a decrease in the mean corpuscular hemoglobin concentration and an increase in fragility and spheroidicity of the red cells.

As early as 1883, Hammarsten reported that the red blood cells in the splenic venous blood were smaller and less flattened (i.e., spherocytic) than those of arterial blood. Banti, '13, noted more fragile erythrocytes and a greater hemoglobinemia in the blood of the splenic vein when contrasted with the artery.

The previously reported studies of comparative splenic artery and vein platelet content are more divergent than the erythrocyte results. Cori, '23, Tsunashima, '28, Howell and Donahue, '37, and Myers, Maingot and Gordon, '26, found higher splenic arterial than venous concentrations of thrombocytes. Holloway and Blackford, '24, and Cumings, '33, have reported either the converse or negligible differences.

Few studies have been directed toward a comparison of the numbers of white blood cells entering versus those leaving the spleen. Watson and Paine, '42, observed that the initial total white counts of splenic arterial and venous samples

did not differ appreciably in one case of primary splenic neutropenia. After epinephrine contraction of the spleen, however, marked mobilization of white cells via the splenic vein occurred. Differential leucocyte studies were not reported. Hirschboeck, '46, in a single splenic arterial and venous blood study done at the time of splenectomy for Felty's syndrome, noted a significant decrease in the venous granulocytic cell content. Peden, '49, asserts on the basis of total counts that his splenic arterial and venous studies in two cases with leucopenia were "essentially identical". It should be noted, however, that when the absolute numbers of granulocytes are calculated in his cases, the splenic arterial granulocyte values actually exceeded the venous values by approximately 40 per cent. Furthermore, in one of his cases the arterial blood contained one gram more of hemoglobin, 500,000 more red blood cells and 100,000 more platelets per cu. mm. than the splenic venous blood.

It was noted in studies by Wright and coworkers, '51, that on the day of surgery, patients with previously chronic peripheral cytopenias were found to have these deficiencies partially corrected. This spontaneous elevation of the immediate preoperative counts probably reflects the psychogenic stimulation of the patient's own adrenal gland, as originally pointed out by Barcroft and Stephens, '27, and the resultant "auto-epinephrine test" may be typical of the phenomena al-

ready described.

In studying a patient with primary splenic neutropenia, Watson and Paine, '42, reported finding a single phagocytic clasmatocyte with engulfed granulocytes, in the splenic venous sample after epinephrine induced contraction. A similar finding of two clasmatocytes, with ingested red blood cells and granulocytes, was seen in the 100 leucocytes differential of the splenic venous blood in a patient with splenic pan-hematopenia.

Wright and coworkers, '51, concluded from the evidence just presented and from the results of their studies of the action of epinephrine on the relative splenic venous and arterial blood counts that the spleen in its relationship to the circulating formed elements of the blood appears to have functional similarities to the lungs in their mediation of the respired air. The blood entering and leaving the spleen under ordinary basal conditions may be likened to the tidal air during quiet respiration. Voluntary complete expiration (reserve air) followed by a deep inspiration (complemental air) measures the vital capacity of the individual. Similarly relaxation of the spleen as obtained with sodium pentobarbital (Lauda, '33), followed by a maximum contraction induced by epinephrine, will reveal the vital capacity of the spleen as a reservoir of sequestered blood elements. Epinephrine stimulation without previously induced relaxation mobilizes the

reserve splenic blood. The residual air represents the irreducible minimum maintained with the pulmonary alveoli in the intact chest and is comparable to the sinusoidal-parenchymal residual blood obtained only by further manual manipulation of an epinephrine contrasted spleen after its surgical removal.

Thus the anatomic spongelike character of the splenic parenchyma may, at least in part, explain the specific relationship which this organ seems to bear to the hypersplenic syndromes. As a matter of actual experience, each of the theoretically predictable disturbances in the circulating blood elements has now been recognized, and the mechanism proved, through appropriate clinical diagnostic and therapeutic tests, to be due solely to splenic dysfunction. This does not, of course, exclude the bone marrow and other organs as also modifying or contributing to the hematologic and clinical picture in a given syndrome, but the primacy of the splenic factor in any specific instance may usually be provisionally established and ultimately confirmed through total extirpation of all splenic tissue, followed by the prompt and permanent correction of all signs and symptoms of the original disease. The acute or chronic selective sequestration and destruction of red blood cells (congenital or acquired hemolytic icterus), platelets (thrombocytopenic purpura hemorrhagica), granulocytes (primary splenic neutropenia) or any

combination of them (including primary congenital or secondary acquired panhematopenia) have all been successfully treated by the surgical removal of abnormally functioning splenic tissue. These clinical and hematologic observations have formed the background for the broad, general, physiopathologic concept of "hypersplenism".

The converse of these syndromes, viz., natural atrophic hyposplenism or surgically acquired asplenism, fortunately is fraught with no known danger to life. A non-symptomatic, panpolycythemia, participated in by all the marrow elements, usually follows removal of the normal spleen--mediated by the elimination of hypothetical splenic inhibitory hormones on marrow hematopoiesis and/ or by the removal of the local reservoir, spherocytoid-accelerating and other premature aging influences of the splenic parenchymal filter on the circulating blood elements (Sodeman, '50).

In spite of the picture just presented as the explanation for the hypersplenic syndromes it doesn't represent the whole picture. The basic mechanism for the hypersplenic phenomena has been attributed to a "hormonal" influence emanating from the spleen and acting to inhibit the delivery of one or more of the essential blood cell elements arising in the bone marrow. This problem will be discussed in more detail later.

The spleen and its vascular sinuses: It has been known for many years that the spleen has the ability to undergo rhythmical

changes in volume (Krumbhaar, '26). It has been repeatedly observed that these changes bear a direct relationship to the circulating cell volume and inevitably attention has been focused upon the nature and character of the circulation which must govern this reservoir activity. The morphological characteristics of the arteries and the veins of the mammalian spleen are well known. Most investigators are in substantial agreement concerning the arterial vascular tree down to the arterial capillary, and from the venous sinuses, or at least the venules, on outward to the large veins. Concerning the intermediary vascular pattern between the arterial capillary and the venous sinus, however, conflicting opinions have appeared in the literature for the past 90 years resulting in descriptions so contradictory that no consensus has been reached to the present day. Briefly, the arterial side of the splenic vascular system may be considered to consist of six distinguishable parts, four of which belong to the artery proper and two to the red pulp: (a) the intratrabecular part of the artery, generally accompanied by the corresponding vein; (b) the main branches arising from the intratrabecular part; (c) the red pulp which immediately surrounds the lymphoid follicles; (d) the vessels supplying the perifollicular red pulp; (e) the "penicillar" arteries; (f) the terminal red pulp (Gall and Maegraith, '50). On the venous side there are the sinusoids, the collecting venules and the intratra-

becular veins. The mode of connection between the arterial and venous sides have not been satisfactorily demonstrated. Knisely, '36, '37, '38, described the relations and activities of the senous sinuses, relating how they separate the blood cells from the fluid of the blood, either by a continuous filtration process, or by what he called a filtering-filling process. In the latter process the sinuses fill with blood, leaking nearly all the fluid to the surrounding extravascular pulp spaces, and packing the cells closely together. The cells are then stored in the sinuses for various periods of time before being released to the venules. Knisely also described long slender capillaries which by-pass the sinuses, and conduct blood from arteriold to venules when the blood cells are being stored in the sinuses. He suggested that these "shunts" nourish the splenic tissue, when the blood flow through the venous sinus route is at a stand-still.

Knisely stated that the various cycles of blood cell storage in the venous sinuses, their filling and emptying, their filtering of cells from fluid, or the rapid conduction of blood from arterial capillary to venule--all these varied activities are precisely controlled by sphincters located at strategic points along the vascular system, in the arteries, arterial capillaries, and at both the afferent and efferent ands of the sinuses.

However, MacKenzie and coworkers, '41, found no evidence

of the cyclical storage activities of the venous sinuses described by Knisely. According to them, the circulation through the spleen is more or less continuous, except for an occasional temporary blockage of a channel by a white blood cell acting like a stopper in a bottle.

The connections between the arterial and venous systems, these authors stated, are pulp channels, the open interstices in the red pulp. They describe the arterial capillaries as terminating in the pulp spaces by a funnel-shaped inlargement, or "ampulla of Thoma". The venous sinuses, similarly, open directly into the pulp interstices. The pulp channel, interposed between an ampulla of Thoma and a venous sinus, may assume the appearance of an intactly walled arteriovenous connection when the spleen is contracted. Thus, the structurally "open" circulation of a relaxed or distended spleen is converted into a functionally "closed" vascular circuit by contraction of the capsule and trabeculae. The factors fundamentally controlling circulatory changes in the spleen are trabecular and capsular contraction, together with arterial constriction. They found no powerful sphincter action anywhere in the red pulp, as Knisely had described.

Recently Peck and Moerr, '51, by using and improving Knisely's method of transillumination of the spleen were able to demonstrate that the intermediary splenic circulation of the red pulp is essentially as Knisely described it, both

structurally and functionally. They were able to see capillaries appear and disappear in great numbers in a given field of the red pulp, that is, for blood flow in them to start and stop, but keep exactly the same pattern hour after hour, up to 10 hours of observation. From this they concluded that they were not haphazard channels in the pulp, but were definite, morphologically intact and integrated components of the vascular system.

Thus in view of the unsettled controversy concerning this portion of splenic circulation it would seem fair to accept as our current working hypothesis the concept that the spleen has a semi-open circulation, controlled by a filter mesh mechanism in the sinus wall, which, under many diverse pathologic conditions, may be altered, in the direction of greater or lesser selective or non-selective permeability to the cellular elements of the blood. This circulatory mechanism, unique as compared with all other organs and tissues, operates to separate the cells from the plasma, and thus to concentrate in the spleen, both within and without the sinuses and in varying degree and quantity, blood cells of various types and quality. The more disturbed the circulatory equilibrium, the more profound and prolonged the stasis, the more does the spleen seem to lack discrimination and to withhold and destroy normal as sell as fragile, senile and damaged elements.

The spleen as a lymphatic organ: The lymphopoietic foci in the splenic parenchyma, made up of typical lymph sinuses, follicles and germinal centers, reflect and parallel the general lymphocytic responses elsewhere throughout the body. It is believed that lymphocytes are normally produced in the mature animal by the malpighian corpuscles, and monocytes may also arise in the spleen (Wintrobe, '51). This lymphatic tissue participates in the hyperplastic reactions in chronic lymphatic leucemia, lymphosarcoma, infectious mononucleosis, serum sickness and other types and varieties of protein sensitizations, and in the hypoplastic inhibition of virus intoxications (Sodeman, '50). The physiologic function or functions of the lymphocyte continue under investigation and discussion. Probably they have to do with endogenous protein metabolism, and possibly with more subtle specific antibody-globulin elaboration or transportation, now believed to be a primary function of the reticulo-endothelial phagocytes. In any event, the characteristic, non-specific, postinfectious peripheral lymphocytoses, and the infiltrative tissue phenomena in localized tubercle formation, tertiary syphilitic lesions and chronic abscesses, are the obvious histopathologic evidence of an important role played by these elements in the body's cellular and humoral defense mechanisms (Sodeman, '50).

The spleen and the reticulo-endothelial system of cells: The spleen is the largest collection of macrophage tissue in

the body. The activity of the phagocytic tissue cells of the body has been extensively studied and their importance in the disposal of invading microorganisms emphasized. The experimental evidence and the evidence from pathological and anatomical studies have clearly demonstrated that injected antigen and particulate matter will accumulate in great concentration in the spleen, because of its sinusoidal structure and its high content of phagocytic elements (Perla and Marmorston, '35). The fixed endothelial cells lining the vascular and lymphatic sinusoids, or anchored in midstream, as in the liver (Kupffer's cells), have a marked potential physiologic, protective phagocytic capacity, in situ and as free, desquamated clasmatocytes or macrophages. The reticulum cells of the adult areolar connective tissues behave as do primitive mesenchymal elements with embryonic potentialities. They give rise to other fixed reticulum cells and to the primitive free cells from which monocytes normally differentiate and mature. In appropriate circumstances this focus of potentially developmental and embryonic-like mesenchymal tissue in the adult spleen may revert to its earlier in utero hematopoietic function and present an essential, compensatory, extramedullary, ectopic center of blood cell formation (Sodeman, '50).

The reticulo-endothelial system is important in the production of antibodies, and splenectomized individuals do

not produce antibodies as well as normal persons (Rowley, '50). The spleen is essential to the maintainence of acquired resistance to certain bacterial, protozoan and hematozoon infections. Cannon and McClelland, '29, showed that the removal of the spleen in albino rats infected with the virus of Bartonella muris led to the development of acute infectious anemia. Adequate blockade of the reticulo-endothelial system with india ink permitted the development of infectious anemia in a manner similar to that observed in splenectomized rats. These authors concluded that the reticulo-endothelial system, and particularly the spleen, functioned to restrain the development of the Bartonella infection; and that interference with the reticulo-endothelial system, either by ablation or by blockade, allowed the development of the virus and the production of the infectious anemia. Splenectomy in certain animal species depresses the natural resistance to acute and chronic infections. The severity of tuberculous infections in mice is increased by splenectomy (Perla and Marmorston, '35).

The macrophages of the reticulo-endothelial system are probably responsible for taking the broken down blood cells from the circulation and they may even store the iron which is to be used in hematopoiesis (Smith and Copenhaver, '48).

The reticulo-endothelial system, as could be predicted, is also involved in a variety of pathological states. The

term reticuloendotheliosis is applied to the systemic, proliferative responses of the embryonal reticulum cells of the body to a stimulating agent. This specific response has been known for a long time in tuberculosis, syphilis, typhoid fever, and has recently been studied in histoplasmosis (Agress and Gray, '39). However the stimulating agent is not known in Hodgkin's disease and in a group of pathological conditions such as Letterer-Sive's disease, Hand-Schuller-Christian's disease, eosinophilic granuloma, and leucemic reticulo-endotheliosis. These conditions have therefore, been widely classified as idiopathic, or infectious, reticuloendotheliosis. Idiopathic reticulo-endotheliosis may take an acute, rapid course, terminating life within a few weeks, or months to give the clinical picture of Letterer-Siwe's disease or may be chronic and extend over many years giving the clinical picture of Hand-Schuller-Christian's disease. The pathological process in these cases, however, shows the same basic pattern: (a) proliferation of large, round monocytic histiocytes, or reticuloendothelial cells, which is accompanied by the appearance of giant cells. Foam cells appear usually during this stage and elements of inflammation such as eosinophilic and polynuclear leucocytes can be seen. The origin of the histiocytes from the reticuloendothelial system can be shown by silver staining which reveals the characteristic fibers; (b) the proliferating cells are short-lived and

spindle-shaped fibroblasts immigrate. During the transition stage, both round histiocytes and spindle-shaped fibroblasts are seen and giant cells may still be found. There is a lack of vascularization in these areas during this phase and cell necrosis and hemorrhage can be observed; (c) fibrosis takes place, connective tissue replaces the areas of granulation, and, after a certain period of time, only scars can be seen in these spots (Freud, '51). The spleen being a major portion of the reticule-endothelial system will show the architectural changes described which are due to proliferation and replacement of the stromal cells.

RELATION OF THE SPLEEN TO BLOOD FORMATION: Hemopoiesis regularly occurs in the embryonic mammalian spleen just as it does in the liver (Krumbhaar, '26). Erythropoiesis in the spleen is at first more pronounced than leucopoiesis but it is short-lived, commencing two months after blood formation appears in the liver and terminating at about the fifth month of fetal life. It may persist to a slight degree until birth. The spleen, however, is chiefly concerned with lymphopoiesis (Gilmore, '41). Except for lymphopoiesis, the adult spleen is not normally concerned with hematopoiesis, this being subject to species differences.

Extramedullary hematopoiesis is found in association with severe anemia, (Brannan, '27). Tumors of such tissue have been observed in the hiluses of the kidney in erythro-

blastic anemia. In pernicious anemia during relapse, nodules of erythrogenic tissue are regularly found in the spleen and liver (Lyall, '35; Meyer and Heineke, '05). In association with macrocytic anemia of other types, and particularly in the macrocytic anemia of liver disease (Wintrobe, '36), these foci are found in the spleen. Similar findings have been noted in osteosclerosis (Donhauser, '08) and in cases with invasion of the bone marrow such as carcinoma (Brannan, '27; Jordan, '42) or Hodgkin's disease (Wintrobe, '36) and even when the anemia was normocytic and not very severe, as well as in erythremia and in cases in which bone marrow hypofunction followed injury by some toxin (Wintrobe, '36). In the last-mentioned type of cases, splenomegaly has often been so pronounced as to suggest a diagnosis of leucemia. Heterotopic bone marrow has been described in hemolytic jaundice (Hartfall and Stewart, '33) and such tumors have been observed in adipose tissue in cases of severe sepsis with anemia (Blaisdell, '33; Brannan, '27). Extramedullary blood formation in the spleen, lymph nodes and liver is a regular occurrence in leucemia (Jordan, '34; Schiller, '43). Experimentally it has been produced by repeated bleeding and by chronic poisoning with blood-destroying substances (Brannan, '27) as well as by the intravenous injection of the cellular constituents of the bone marrow (Osogoe and Omura, '50). As was pointed out in the introduction, ectopic erythrocytopoiesis as well

as megakaryocyte formation developed so rapidly and so effectively in the spleens of mice injected with radioactive strontium that no anemia developed, in contrast to their splenectomized brothers (Jacobson, Simmons and Block, '49). This indicates that this organ remains a potential source of blood formation.

The nodules of blood-forming tissue may be composed of normoblasts or their precursors, granuloblasts, megakaryocytes, or of all three strains of cells. It is a moot point whether these cells arise from lymphocytes in the blood, as the unitarians hold (Jordan, '27), or from undifferentiated hemocytoblasts, or by an alteration of clasmatocytes or other partially or completely differentiated cells. Most hematologists hold that extramedullary hematopoiesis is a compensatory phenomenon and the readiness with which this change occurs in infants and young children, in whom the bone marrow has little or no room for expansion is cited as evidence (Brannan, '27).

THE ENDOCRINE ACTIVITIES OF THE SPLEEN: The possibility that there is in the spleen some diffusible substance which might exert a hormonal-like effect was suggested, among others, by Lauda in 1933 when he observed a reduction of the bleeding time following the injection of extract of spleens. Perhaps the first detailed experimental attempt along this line was that of Torrioli and Pusic, '34, who injected large doses of

a protein-free aqueous extract of a normal spleen intravenously into rabbits and thereby greatly reduced the peripheral blood platelet count. Earlier work by Torrioli and Puddu, '34, demonstrated that splenic extracts prepared from the spleens of patients with idiopathic thrombocytopenic purpura injured in vitro megakaryocyte cultures of the bone marrows of guinea pigs. During the succeeding years (1938 to 1942) many attempts were made to corroborate or negate these findings.

In spite of a great deal of circumstantial evidence, there was no valid proof of an internal secretion of the spleen. In fact, the endocrine glands per se were known to have a variable effect on the destruction of thrombocytes in the peripheral blood (Tocantins, '38); e.g., in rats orchiectomy and ovariectomy are followed by thrombocytopenia (Bankow, '31), while after either adrenalectomy or the injection of extracts of sexual glands, there is thrombocytosis (Shecket, Friedman and Nice, '35). Many inconclusive arguments have been proposed suggesting hypersplenism on a hormonal basis as the causative factor in the production of the platelet depression associated with idiopathic thrombocytopenic purpura. For example, many surgeons have made the observation that at the time of splenectomy the abnormal oozing of blood frequently stops abruptly when the splenic

pedicle is clamped in spite of the fact that the number of thrombocytes in the peripheral blood remains unchanged at that time; therefore, the effect on hemostasis could be due to some substance or substances in the blood other than the platelets. In fact, according to Nickerson and Sunderland, '37, thrombocytopenia is not the sole factor in the causation of hemorrhages. These authors are unable to say whether there is a concomitant qualitative defect of the platelets or a capillary hyperpermeability or both. However, the correlation between the bleeding tendency and the platelet level is so close one feels that the thrombocyte must indeed be at least one important factor in the purpuric manifestations of Werlhof's disease.

Up to the present time seventeen papers have been published in the English language in which ten investigators (Troland and Lee, '38; Hobson and Witts, '40; Otenasek and Lee, '41; Rose and Boyer, '41; Paul, '42; Cronkite, '44; Moolten, '45; Singer and Rotter, '49; Dameshek and Estren, '50; Platt and Zeller, '51; Harrington, Minnich, Hollingsworth and Moore, '51) have demonstrated the presence of a diffusible substance obtained from acetone extracts of spleens of patients splenectomized because of diagnosed idiopathic thrombocytopenic purpura. Six other workers (Pohle and Meyer, '39; Major and Webber, '39; Tocantins, '39; Hodge

and Strong, '39; Wiseman, Doan and Wilson, '40; Colmer and Mersheimer, '41) obtained negative results. Uihlein's, '42, findings were variable and inconclusive.

Platt and Zeller, '51, were of the opinion that the detailed chemical extraction and bioassay of splenic tissue by Moolten, '45, as well as the significant histological findings, seem to favor hypersplenism conclusively as the basis for the thrombocytopenia occurring in cases of Werlhof's disease. Moolten demonstrated that two steriod preparations exist in the spleen in comparative abundance and may be isolated in relatively concentrated form absorbed on cholesterol following crystallization of the latter from crude acetone-ether extracts, previously emulsified and dissolved in absolute methyl alcohol; one, a platelet-lowering lipid, was called "thrombocytopen"; the other, a platelet-elevating lipid, was called "thrombocytosin." An analysis of the bioassayed material suggests tentatively that in the human organism significant amounts of both thrombocytopen and thrombocytosin occur in the spleen, diminishing in amount with metaplasia or with replacement of its functioning reticulo-endothelial elements (hemosiderosis, Hodgkin's disease, leucemia). It therefore appears that the spleen exerts a dual control on the rate at which platelets are delivered into the circulating blood. Furthermore, after several days' administration of large doses of thrombocytopen a refractory state and "platelet

escape" is observed (Platt and Zeller, '51). Simultaneously, the bone marrow reveals a remarkable hyperplasia of megakaryocytes, which are in various states of maturation.

Histologically, in purpura hemorrhagica the spleen may exhibit lipoidal vacuolation of the hyperplastic reticulum cells. When this microscopic pattern predominates thrombocytopen may be extracted in great quantity. On the other hand, thrombocytosin is apparently stored in subcutaneous adipose tissue, and its mobilization from traumatized fat cells may possibly explain elevations in thrombocyte count after surgical operations in general (Adams, '44). Both factors are also present in egg yolk, thrombocytosin predominating and effective orally. The purified factors are apparently nontoxic, thrombocytosin being tentatively classified as a fat-soluble dietary factor and thrombocytopen as a fat-soluble hormone (Platt and Zeller, '51).

In addition to the platelet reducing substance, "thrombocytopen", there is considerable evidence to support a theory of splenic hormonal activity directed towards the bone marrow. These controlling factors apparently are inhibitory in character. Such a role is suggested by changes which have been noted frequently following splenectomy; namely leucocytosis, increase of reticulocytes, appearance of Howell-Jolly bodies and nucleated red corpuscles, and increase in platelets. Actual cellular hyperplasia in the bone marrow may occur

(Krumbhaar, '32). In the splenectomized dog made anemic by bleeding or by acetylphenylhydrazine injections, the number of normoblasts in the peripheral circulation is four times greater than in the non-splenectomized animal and the number of primitive erythroblasts is much higher (Cruz and Robscheit-Robbins, '42). It has been shown that the leucocytosis which follows splenectomy in rats fails to develop in parabiotic animals until the second partner has been splenectomized. (Palmer, Kemp, Cartwright and Wintrobe, '51). All of these observations can be interpreted as indicating that the spleen inhibits the liberation of cells from the marrow. This inhibition, under normal conditions, is advantageous presumably, since the appearance of immature cells in the peripheral blood may be regarded as evidence of lessened efficiency.

Dameshek and Estren, '50, after reviewing the literature concerning pathological splenic activity summarized that hypersplenism is a functional diagnosis indicating hyperactivity of the spleen and resulting in various effects on the blood cells. Whenever the spleen becomes anatomically enlarged--whatever the cause for the enlargement--it may become physiologically hyperactive. The results of this hyperactivity are largely inhibition of production and delivery of cells at the marrow.

Jacobson, Simmons, Marks, Gaston, Robson and Eldredge, '51, made the extremely interesting observation that lead-

protection of the marsupialized spleen from irradiation applied otherwise to the total animal, makes possible a rapid return to normal hematopoiesis in the bone marrow and protects the animal from total marrow aplasia and death. As to whether the factor or factors involved in this protective action are due to cell migration from the shielded tissue or dispersion of a non-cellular substance these authors could only speculate. However, they favored explaining their experimental results on the basis of a humoral non-cellular substance which stimulated regeneration of the hematopoietic tissue or supplies something which makes repair and regrowth possible. This will be discussed at more length in the evaluation of the findings being reported in this paper.

Thus far evidence has been presented which tends to support both of the concepts on splenism, i.e., (a) that the function of the normal spleen is primarily phagocytic, that is, the removal of aged red cells, white cells and platelets from the circulation, (b) that its function is primarily regulatory with particular reference to the formation and delivery to the blood of red cells, white cells, and platelets of the marrow. No attempt has or will be made to disprove concept. It seems apparent from the foregoing review of the literature that both concepts are probably correct in at least some measure and the author has accepted this as the only reasonable explanation of the known, demonstrable phen-

omenona relative to the spleen.

However, the relatively unexplored factors of the spleen or possibly the entire reticulo-endothelial system which are concerned with protection from radiation injury represent possibilities which deserve considerable probing. An effort has been made to determine what phenomena are present when the bone marrow has been injured by radiophosphorus but the spleen present and able to compensate and again what further effects radiophosphorus has on the bone marrow after the animal has been splenectomized. Understanding these phenomena will inevitably lead to a clearer understanding of splenic functions in general.

MATERIAL AND METHODS

Five series of animals were used in this particular project, all of the animals being obtained from the albino rat colony which the Department of Anatomy has maintained and inbred since 1925. The first series was composed of nine animals, seven males and two females, and served as the control series for the determination of a range of normal values so that deviations from this range in the experimental series could be evaluated. The average values for peripheral blood counts and bone marrow differential counts from this series are compared with similar values which had been determined previously by various workers in the Department from animals of this same colony.

The second series consisted of eight animals all of which were males. These animals were treated with 1.0 microcurie radioactive phosphorus per gram of body weight and then sacrificed at two, four, six, eight, eleven, fourteen, seventeen and twenty day intervals respectively after the treatment. In all of the animals treated with radioactive phosphorus the material was administered intraperitoneally in the form of dilute sodium phosphate in distilled water; in nearly every case less than on cc. of material was injected into each animal. No deaths occurred as a result of improper administration. All of the animals were anesthetized with ether before they were injected and little heed was paid to

aseptic technique.

The radioactive phosphorus was obtained from U.S. Atomic Energy Commission, Oak Ridge, Tennessee and is considered to be carrier free. New shipments are received by the Department of Radiology once every two months and are assayed against a Bureau of Standards radium D plus E standard at the time of arrival. This material is then diluted to 400 microcuries per cc. The activity in microcuries per cc. at the time the material was injected into the experimental animals was determined by substituting the time which had elapsed since the assay in the following formula:

$$N = N_o e^{-\lambda t}$$

Where: N is the activity to be determined.

N_o is the activity at the time of assay and dilution.

λ is the decay constant for radioactive phosphorus and has been determined to be 5.61×10^{-7} sec.$^{-1}$.

t is the time in seconds since the assay and dilution to 400 microcuries per cc.

The radiations from radioactive phosphorus are moderately soft beta rays with an average mev of 0.7; they are completely absorbed by eight mm. of tissue, and fifty per cent absorbed by three mm. of tissue.

All of the animals in the next three experimental series, with one exception, underwent splenectomy. The one exception was subjected to the same procedure as the others except for

the actual ligation of the splenic vessels and removal of the spleen. To obtain a sufficient number of splenectomized experimental animals four different groups were splenectomized by the following procedure: A two inch midline incision was made into the peritoneal cavity and the spleen and its pedicle were exteriorized. The vessels in the pedicle were then ligated, the pedicle divided and the spleen removed. The peritoneum and abdominal muscles were sutured with interrupted cotton sutures and the skin was sutured with a running mattress cotton suture. Ether was used to produce anesthesia and asepsis was only minimally observed.

The first group of animals to be splenectomized was composed of eight males all of which died within a week after surgery giving a mortality of 62.5 per cent. The other three recovered and were subsequently used as experimental animals. All were to be injected with radioactive phosphorus either forty-seven or forty-eight days after splenectomy but one died an anesthetic death so was used as a control for the splenectomized groups. Unfortunately no peripheral blood studies were made on this animal. The other two animals of this group were injected with 1.0 microcurie radioactive phosphorus per gram of body weight and subsequently sacrificed at eleven and seventeen days respectively after treatment with radiophosphorus or fifty-eight and sixty-four days respectively after undergoing splenectomy.

The third group of animals to be splenectomized was comprised of eight males two of which died within a week after surgery giving a mortality of only 25 per cent. Two of the animals served as control splenectomized animals and were sacrificed at eighteen and twenty-eight days after splenectomy respectively. The four remaining animals were treated with 1.0 microcurie radiophosphorus per gram of body weight eighteen days after splenectomy and subsequently sacrificed at two, six, fourteen and twenty days after treatment with radiophosphorus, or twenty, twenty-four, thirty-two and thirty-eight days respectively after splenectomy.

The fourth group consisted of three splenectomized animals and the one exception which underwent laparotomy but not removal of the spleen. All of these animals were females and all recovered from the surgery. One of the splenectomized animals served as a control and was sacrificed at thirty-eight days after surgery. Peripheral blood studies were made before surgery on the other two splenectomized animals in this group and also on the animal which underwent laparotomy. These studies were repeated at four days, eight days, fourteen days and the animals were sacrificed at twenty days after surgery.

For purposes of clarity in reporting and comparison of findings the experimental animals which had under gone surgery have been arranged into three series, i.e., series three,

four and five. Series three consisted of three experimental animals all females. Peripheral blood studies were made on all three just prior to surgery and then again four, eight, fourteen and twenty days after surgery at which time they were sacrificed. Two of the animals had undergone splenectomy and the third laparotomy as previously described.

Series four consisted of four experimental animals, two males and two females, all of which had undergone splenectomy. The two males were sacrificed at eighteen and twenty-eight days respectively and the two females were sacrificed at thirty-eight and forty-eight days respectively after splenectomy.

Series five was composed of six experimental animals all of which had been splenectomized and all of which were treated with 1.0 microcurie radioactive phosphorus per gram of body weight. The animals were sacrificed at two, six, eleven, fourteen, seventeen and twenty days respectively after treatment with radiophosphorus, or twenty, twenty-four, fifty-eight, thirty-two, sixty-four and thirty-eight days respectively after splenectomy. The animals which were sacrificed at eleven and seventeen days after treatment with radiophosphorus or fifty-eight and sixty-four days respectively after splenectomy were female. All other animals in series five were males.

The following determinations were made on the peripheral

blood: Total red cell count, total white cell count, hematocrit, reticulocyte count, and differential leucocyte count. A Bright Line hemocytometer was used for both the total erythrocyte counts and the total leucocyte counts. Heyem's diluting fluid was used for the rythrocyte counts and three per cent acetic acid as the diluting fluid to lake the red cells for the leucocyte counts.

Sufficient blood for the hematocrit determination was obtained from the tail of each rat and immediately oxalated. A Wintrobe hematocrit tube was filled with this oxalated blood, the tube placed in a centrifuge machine and spun at 2500 rpm for thirty minutes to determine this value.

For the reticulocyte count brilliant cresyl blue dissolved in absolute methyl alcohol was spread as evenly as possible on a slide and allowed to dry. A small drop of fresh blood was placed on a cover slip which was then placed on the dried film of brilliant cresyl blue drop side down with enough pressure applied to insure an even spreading of the frop of blood. This was allowed to dry and the number of erythrocytes containing a reticulum was determined per 1000 erythrocytes.

Fresh, whole blood was smeared evenly on cover slips, allowed to dry, and then stained with Wright's stain followed by Giemsa's blood stain for the determination of the differential leucocyte count. Three hundred consecutive white cells

were counted and differentiated for this determination.

After adequate blood had been obtained for the previously described determinations, the animals, which had been under ether anesthetic all of this time, were given a heavier dose of ether to insure death. Just before death occurred, the animals were dissected and the axillary lymph nodes, a portion of the liver, both femurs and the spleen (if not previously removed) were removed. The lymph nodes, liver and spleen were placed in fixer--either Zenker formol or picro-aceto-formol. The shafts of both femurs were splet longitudinally and the marrow of one femur was scooped out, this marrow being used to make imprints on cover slips. The marrow in the other femur was allowed to remain in the split cortical halves. These latter were then fixed in the same manner as the lymph nodes, liver, and spleen.

The marrow imprints were allowed to dry and then were stained as the peripheral blood smears had been stained--with Wright-Giemsa blood stain. A differential count of the imprints was subsequently made, five hundred cells being counted for each determination.

Sections of lymph node, spleen, liver, and bone marrow were made, mounted on slides, and stained with hematoxylin and eosin or hematoxylin-axure II-eosin. This material was later examined to determine the normal appearance of the structures and what variations were present in the experimental material.

RESULTS AND OBSERVATIONS

CONTROL SERIES (SERIES I)

Peripheral blood: The picture of the peripheral blood as determined in animals of the stock of the Department of Anatomy is given in table I.

It can be said that in this stock the total erythrocyte counts have normally varied between 8.0 and 10.4 millions and leucocyte counts between 12 and 20 thousands. The neutrophilic percentage has ranged around 20 per cent, the lymphocytic around 75 per cent, eosinophils 5 per cent, the monocytes 5 per cent. These values compare favorably with those found for the control animals used in this work.

Bone marrow: Normally active hematopoietic tissue fills the femoral shaft of the rat. The hematopoietic cells are contained within a loose reticular network and the few intervening spaces are occupied by fat cells and blood sinuses (fig. 1). The average granuloid/erythroid ratio for the control animals used in this work is 1.28/1.00 with variations ranging from 0.85/1.00 to 2.57/1.00. This is slightly lower than the 1.47/1.00 found by Latta and Nelson, '48, and slightly higher than the 1.17/1.00 found by Imes, '40, both of whom had previously worked with animals from this same stock. The average of differential counts for three control animals given by Imes, the average of differential counts for three control animals given by Nelson and the average of differ-

	Latta-Ehlers 1931	Latta-Moore 1932	Latta-Benner 1934	Latta-Henderson 1937	Imes 1940	Latta-Nelson 1948	Author's
Number of Animals	4	8	22	29	20	20	9
Total RBC (Millions)	7.9 7.4 - 8.5	8.2 6.8 - 9.4	9.4	9.1	10.4 6.8 - 12.4	8.9 7.9 - 10.1	9.4 8.3 - 10.3
Total WBC (Thousands)	15.8 11.2 - 19.9	16.9 6.3 - 24.9	19.7 12.7 - 26.0	18 - 20	12.6 7.3 - 28.4	13.8 7.6 - 17.2	15.0 8.4 - 26.2
Lymphocytes	73 % 69 - 82	72 % 59 - 78	74 %	74 %	71 % 51.5 - 84	82.2 % 74 - 91.5	74.9 % 61.5 - 83.5
Neutrophils	16 % 8 - 22	29 % 17.8 % 6.5 - 26	20 %	16 %	23.4 % 11 - 44	15.6 % 5.5 - 23 10 % 4 - 15.5	18.3 % 12.5 - 28.5 16.4 % 11.5 - 33.0
Segs					22.5 % 9 - 44		
Staff		0.5 % 0.5 - 2			0.8 % 0 - 2	6.3 % 1 - 12	1.9 % 0 - 6.0
Juveniles		0.8 % 0.5 - 1			0.11 % 0 - 1	0	0
Eosinophils	5 % 1 - 7	6.3 % 0.5 - 6	2 %	4 %	2 % 0 - 5	2.1 % 0 - 6	3.8 % 0 - 8.5
Basophils					0.34 % 0 - 1.5		0.3 % 0 - 1.5
Monocytes	6 % 3 - 8	6.1 % 2 - 9	4 %	6 %	3.1 % 0 - 15	1 % 0 - 3.5	2.1 % 0.5 - 4.0
Reticulocytes		1.6 % 0.9 - 2.4	2.9 %			2.4 % 0.6 - 4.7	2.9 % 1.6 - 4.3

Table I. Blood counts from normal rats from the stock of the Department.

ential counts for eight control animals used herein are found in the following tabulation:

Cell types	Imes	Nelson	Author's
Neutrophils	44.0	47.0	40.4
Segmented	8.5	1.9	12.5
Staff	16.2	29.0	14.9
Juvenile	13.0	10.3	8.5
Myelocyte	6.3	6.3	4.7
Eosinophils	8.1	6.6	9.8
Segmented	---	---	0.4
Staff	1.6	5.2	2.5
Juvenile	6.5	1.1	3.9
Meylocyte	---	0.3	2.8
Basophils	---	---	1.2
Promyelocytes	1.1	1.3	2.4
Hemoblasts	0.2	0.9	1.0
Erythroblasts	46.1	37.3	45.3
Basophilic	0.3	0.9	9.7
Polychromatic	9.5	13.3	15.6
Orthochromatic	36.3	23.6	20.1

These averages seem to compare fairly well with the mean values of Stasney and Higgins, '35, who found a granuloid/erythroid ratio of 1.75/1.00

Spleen: The rat spleen, as other mammalian spleens, consists of two regions--the red pulp and the white pulp. The white pulp is lymphatic tissue forming a more or less continuous sheath around the branches of the arterial system expanded into nodules which contain germinal centers eccentrically placed with respect to the arterioles. The nodules of white pulp contain two zones, the inner zone containing densely packed lymphocytes and the outer zone populated almost exclusively by medium sized lymphocytes. The outer zone merges gradually with the red pulp. The red pulp consists of cords

of reticular cells separating the venous sinuses of a rich vascular bed. Among these cords and in the sinuses are all of the elements of the circulating blood, but with a higher proportion of lymphocytes. The red pulp is also the site of formation of all types of blood cells normally formed in the marrow (fig. 2).

Lymph nodes: The rat lymph nodes also are similar to other mammalian lymph nodes. These are covered by a very definite capsule of connective tissue. At its outer surface the capsule blends with the surrounding connective tissue and in this way the organ is attached in position. The capsule consists of rather closely packed bundles of white fibrous connective tissue and scattered elastic fibers, which are more numerous in its inner layer. A few smooth muscle fibers can be found in the capsule around the points of entrance and exit lymphatic vessels. At the hilum there is a depression where the capsule is thickened and extends deep into the node. At various points over the surface of the node the capsule gives off septa or trabeculae which extend into the substance of the organ. Both the trabeculae of connective tissue and the elements of the lymphoid tissue are arranged differently in the outer or cortical and the inner or medullary part of the node.

Liver: In the rat, as in other mammals, the liver is a compound tubular gland, situated in the upper and right part of the abdominal cavity, immediately below the diaphragm.

Several fissures partially divide it into four lobes: (a) a median or cystic lobe, bearing a deep fissure for the hepatic ligament; (b) a right lobe partially divided into anterior and posterior lobules; (c) a large left lobe; and (d) a small caudate lobe which lies deep and fits around the esophagus. Since a gall bladder is absent in the rat, tributary ducts from the various lobes unite to form the bile duct or ductus choledochus. It is incompletely invested by an outer tunica serosa, derived from the peritoneum, within which is a delicate connective tissue capsule. This capsule, which contains a fair abundance of elastic fibers, covers the entire surface of the organ. At the transverse fissure, it surrounds the entering blood vessels and follows into the gland, forming a framework and dividing it into innumerable small lobules. The lobules are cylindrical or irregularly polyhedral in shape and approximately one mm. in breadth and two mm. in length. Excepting just beneath the capsule, where they are frequently arranged with their apices directed toward the sufface, the lobules are irregularly arranged.

The hepatic lobule, which may be considered the anatomical unit of structure of the liver, consists of anastomosing secreting tubules, the hepatic cords or plates, radially arranged about a central blood vessel which traverses the length of the lobule.

RADIOACTIVE PHOSPHORUS TREATED, NON-SPLENECTOMIZED SERIES

(SERIES II)

Peripheral blood: In these animals which were treated with 1.0 microcurie radiophosphorus per gram of body weight, there was a depressed leucocyte count ranging from 4,400 cells/cu.mm. in the animal sacrificed on the seventeenth day to 9,200 cells/cu.mm. in the animal sacrificed on the twentieth day. The average count for the animals which had received this dosage with their spleens intact was 6,600 cells/cu.mm. as compared to an average of 15,000 cells/cu.mm. for untreated non-splenectomized rats (tables I and II). Normal differential counts were found for the animals of this series (table II). However, seven of the eight animals in this series (a total leucocyte count was not obtained from the animal sacrificed eleven days after treatment) showed a significant leucopenia. Therefore the absolute numbers for both circulating granulocytes and circulating lymphocytes must have been reduced by the same degree.

There was an unmistakable depression of the reticulocyte count in six of the eight animals of this series. Peripheral blood was not obtained from the animal which was sacrificed on the eleventh day after treatment because of technical difficulties and the animal of this series which was sacrificed on the seventeenth day after treatment had a reticulocyte count three and a half times the average seen in the other animals

of this series (table II). This, it was concluded, was evidence of a reticulocyte shower.

In contrast to the alterations in the reticulocyte counts, however, none of the total erythrocyte counts for the animals of this series were low enough to be suggestive of an anemia with the exception of the animals which were sacrificed on the sixth and the twentieth days after treatment (table II). After studies on the animal which was sacrificed twenty days after being treated with 1.0 microcurie per gram of body weight were completed, it was speculated as to whether the anemia was the result of radiation injury and would become progressively more severe, whether this represented the culmination of damage and following this there would be evidence of regeneration or whether this was just a normal variation peculiar to this particular animal as was assumed to be the case for the animal sacrificed six days after treatment. Subsequent work in the Department of Anatomy has indicated that this was most likely a combination of both a normal variation and cumulative damage. This work has pointed out that a reticulocytosis of the same order as the depression seen for this series becomes evident twenty days and longer after treatment (Latta and Kamprath, '51). This could be taken to mean that the concentration of radiophosphorus in the bone after the injection of 1.0 microcurie per gram of body weight up to twenty days is great enough to inhibit the maturation and delivery of

Days after Treatment	2	4	6	8	11	14	17	20
Lymphocytes	76.0	63.0	67.0	62.0	82.0	70.0	64.0	75.0
Neutrophils	18.5	33.0	28.5	34.5	16.5	23.0	33.5	21.5
Segs	17.0	32.5	26.0	34.0	15.5	20.5	29.5	19.0
Staff	1.5	0.5	2.5	0.5	1.0	2.5	4.0	2.5
Juveniles	0	0	0	0	0	0	0	0
Eosinophils	2.0	2.5	1.5	0.5	0	1.5	0	0.5
Basophils	0	0	0	0	0	0	0	0
Monocytes	3.5	1.5	3.0	2.5	1.5	5.5	2.5	3.0
W.B.C.	6.6	6.3	6.7	7.4	-	5.6	4.4	9.2
R.B.C.	8.8	9.7	7.3	9.2	-	9.2	8.4	7.7
Reticulocytes	0.7	0.5	0.7	1.5	-	1.5	3.7	0.6

Table II. Differential leucocyte percentages, total leucocyte counts in thousands/cu.mm., total erythrocyte counts in millions/cu.mm. and reticulocyte percentages for the animals of the non-splenectomized radiophosphorus-treated series.

reticulocytes as well as granulocytes to the circulating blood, but that following this time the concentration falls below the point necessary to prevent regeneration. The variations from animal to animal, however, preclude the possibility of interpreting the findings with accuracy.

Bone marrow: Differential counts made from marrow imprints showed a very definite progressive drop in the granuloid/erythroid ratio from 2.22/1.00 to 0.23/1.00 in the animals sacrificed between the two day interval to the fourteen day interval following treatment. The G/E ratio had increased to 0.77/1.00 in the animal sacrificed seventeen days after treatment and in the animal sacrificed twenty days after treatment with 1.0 microcurie per gram of body weight there had been a very marked rise to 5.58/1.00 (table III). Subsequent work (Latta and Kamprath, '51) has again shown that a rise to nearer normal levels occurs in the G/E ratio by the twentieth day but that such a sharp increase of granuloid elements over erythroid elements as was seen in the animal of this series studied at the twenty day interval is not a normal or consistant finding.

The marrow imprint at the fourteenth day interval revealed a large preponderance of mature erythroblasts (fig. 3). This preponderance was not so marked in the animals sacrificed on the seventeenth day after treatment but it should be pointed out that this animal also showed a relative reticulocytosis over the other animals in this series (table II).

Days after Treatment	2	4	6	8	11	14	17	20
Granulocytic Cells	69.0	55.2	49.8	34.6	29.6	18.2	43.4	84.8
Neutrophils	59.5	47.8	40.6	30.4	23.2	13.4	39.4	76.6
Segs	31.8	20.2	12.2	10.4	4.4	3.0	10.4	23.0
Staff	14.0	16.0	16.8	8.8	8.2	4.4	13.0	32.2
Juveniles	7.8	5.4	6.7	6.2	6.4	3.0	7.0	14.6
Myelocytes	6.0	6.2	5.0	5.0	4.2	3.0	9.0	9.8
Eosinophils	5.4	3.4	5.2	1.2	3.6	0.6	0.6	2.7
Segs	0.2	0	0	0	0.2	0	0	0
Staff	0.6	0.8	0.4	0.2	1.4	0	0	0.2
Juveniles	2.0	1.4	2.4	0.2	1.0	0.4	0	1.4
Myelocytes	2.6	1.2	2.4	0.8	1.0	0.2	0.6	1.0
Basophils	1.4	1.0	1.0	0.2	0.8	1.4	0.8	0.2
Promyelocytes	2.2	2.8	2.0	1.6	1.8	1.6	2.2	2.0
Hemoblasts	0.4	0.2	1.0	1.2	0.2	1.2	0.4	0.4
Erythrocytic Cells	31.0	44.8	50.2	65.4	70.4	81.8	57.6	15.2
Basochromes	7.2	4.8	6.4	4.4	5.8	11.4	3.6	6.8
Polychromes	10.8	11.0	8.6	22.6	12.0	19.4	9.0	3.8
Orthochromes	13.0	29.0	35.2	38.4	52.6	51.0	44.0	4.6
Granuloid/Erythroid Ratio	2.22/ 1.00	1.23/ 1.00	0.99/ 1.00	0.53/ 1.00	0.42/ 1.00	0.23/ 1.00	0.77/ 1.00	5.58/ 1.00

Table III. Values obtained from differential counts of touch preparations of femoral marrow from the animals of the non-splenectomized radiophosphorus-treated series.

Sectioned specimens from the marrow of the animal in this series sacrificed on the fourteenth day after treatment (fig. 4) showed congestion and loss of the normal stromal pattern. There were many mature red blood cells found in the stromal areas outside of the sinusoids and blood vessels. The loose texture of the cellular elements gave the impression of hypoplasia but there was little evidence of an increase in the number of sinusoids and fat cells. There was, however, a relative increase in the number of megakaryocytes to be seen in the marrow, and also the number of erythropoietic foci in relation to the granulopoietic tissue was increased over that seen in control sections. The damage seen on the fourteenth day post treatment is fairly typical of all of the marrow removed from all of the animals of this series up to seventeen days after treatment but is somewhat more severe. Sectioned material from the animal from this series sacrificed at the twenty day interval revealed striking regeneration of the granuloid elements (fig. 5). That such a regeneration occurs has been supported by other work in the Department (Latta and Kamprath, '51) but that the usual response is not nearly so marked. The point that I wish to make is that granuloid regeneration probably does not occur at the expense of the erythroid population as the material from this particular animal would lead one to think.

It would appear then that both erythroid elements and

granuloid elements were damaged, destroyed or inhibited by this dosage of 1.0 microcurie per gram of body weight. The G/E ratio of 2.22/1.00 for the animal sacrificed at the two day interval would indicate that early the erythroblasts suffered more than the granuloblasts. If this is true, it must be concluded from the G/E ratios of the animals sacrificed from the fourth day to the seventeenth day that while the myeloid elements continued to be depressed the erythroid elements were actively regenerating. The number of more mature erythroblasts progressively increased in the marrows up to the fourteenth day interval but in the face of a depressed reticulocyte count in the peripheral blood. At the seventeenth day interval concomitant with a relative reticulocytosis the number of late stage erythroblasts had decreased. This seemed as though a wave of erythroblastic regeneration had reached its crest and then fallen and at the twentieth day interval the granuloid elements were actively regenerating pushing erythropoiesis, relatively, into the background.

Although there was not a conspicuous anemia produced, the leucopenias, the depressed reticulocyte counts and the "shift to the right" of the erythropoietic tissue up to fourteen days after treatment in the animal of this series indicate that there apparently had been a strain put on the hematopoietic systems of these animals for which the bone marrows were not completely able to compensate. This same contention

is also supported by the fact, as will be pointed out later, that the spleens of these animals had become more active in myelopoiesis.

Spleen: Consistent changes were found in all of the spleens removed from the animals of this series which were treated with 1.0 microcurie radiophosphorus per gram of body weight. It was possible to demonstrate an increase in the number of erythropoietic foci in the red pulp over the number usually seen in the spleens from untreated animals. These changes (fig. 6) were in evidence at two days and persisted to twenty days after treatment, the end of the experimental period.

There was no evidence of damage to the lymphocyte population which would be expected to be the first sign of any direct radiation injury to the spleen. It was concluded, therefore, that these changes were probably not directly related to the effects of the radiophosphorus but were more likely secondary to the damage seen in the bone marrow. Also this absence of evident damage to the lymphocyte population did not seem in keeping with the finding of a lymphopenia in the animals which were treated with 1.0 microcurie per gram of body weight. Another explanation for the destruction of lymphocytes other than direct damage to the lymphopoietic organs should be sought.

Bloom, '48, also found that ectopic hematopoietic activity was definitely elevated in the spleen of the rat at all

intervals from three to 120 days after in injection of over one half of an LD-50 dose which is 4.5 microcurie per gram of body weight. He found that erythropoiesis was particularly active from five to twenty-five days. This would seem to indicate that in the rat the spleen is very capable of taking over erythropoietic and granulopoietic functions which the damaged bone marrow is no longer sufficiently able to preform.

Lymph nodes: There were no detectable changes in the lymph nodes which could be in any was attributed to the action of the radioactive phosphorus. Since all of the nodes examined were axillary nodes, and also since it is known that radiophosphorus affects lymphatic tissue in different degrees in different parts of the body (Koletsky and Christie, '51), it is possible that damaged areas might have been missed. This would explain the presence of a lymphopenia in the animals treated with 1.0 microcurie per gram of body weight. However, Bloom, '48, using dosages up to more than twice this amount was unable to observe damage in lymphatic tissue regardless of its source. This fact was surprising to him because radioautographs taken ten hours after treatment showed considerable radioactivity in the germinal centers.

Liver: Here, also, no effects were seen at any of the interval studied, this being in complete agreement with previous work with radioactive phosphorus (Platt, '47; Bloom, '48). Even after lethal doses of X-irradiation have been administered

to rats there are no demonstrable lesions in the liver. However, indirect damage can be inflicted on the liver cells by the production of a radiation anemia with its associated anoxia.

SPLENECTOMY-LAPAROTOMY CONTROL SERIES (SERIES III)

As was described in the section on materials and methods, this series consisted of three animals. Two of these animals underwent splenectomy and the other underwent just a laparotomy. Peripheral blood studies were made on all of these animals just prior to surgery and then at intervals of four, eight, fourteen and twenty days after surgery. All of the animals were sacrificed at twenty days after surgery.

Peripheral blood: The peripheral blood picture for the two splenectomized animals were very similar over the twenty day period and will be described together. The total leucocyte counts, erythrocyte counts, reticulocyte counts and differential counts were within normal limits just prior to surgery in both of the splenectomized animals of this series (table IV). At four days post surgery the total erythrocyte counts had dropped an average of 5.5 million cells/cu.mm. to give an average red cell count of 2.4 million cells/cu.mm. for the two animals. The reticulocyte counts for both animals had more than doubled at this time to give an average value of 8.6 per cent. The total leucocyte counts had risen in both animals but more precipitously in one than the other. The

Days after Splenectomy	prior	4	8	14	20
Lymphocytes	79.7	49.7	72.3	77.7	85.3
	83.7	46.3	68.0	76.0	71.7
Neutrophils	12.7	47.3	23.7	15.7	9.7
	11.7	49.0	27.3	20.3	22.7
Segs	12.3	43.0	17.3	15.0	7.3
	10.0	41.0	19.7	19.0	19.3
Staff	0.3	4.3	5.0	0.7	2.3
	1.3	6.7	6.3	1.3	2.7
Juveniles	0	0	1.3	0	0
	0.3	1.3	1.3	0	0.7
Eosinophils	4.3	0.3	2.7	0.3	1.7
	2.7	2.7	1.7	1.7	3.3
Basophils	0	0	0	0	0.3
	0	0	0	0	0
Monocytes	3.3	2.7	1.3	6.3	3.0
	2.0	2.0	3.0	2.0	2.3
W.B.C.	16.3	29.0	40.4	9.0	14.0
	12.9	16.4	41.8	13.0	20.1
R.B.C.	7.8	2.0	2.8	5.2	4.8
	8.5	2.8	2.1	5.2	2.9
Reticulocytes	4.5	10.9	42.6	23.5	11.9
	3.0	6.4	58.9	12.7	44.1

Table IV. Differential leucocyte percentages, total leucocyte counts in thousands/cu.mm., total erythrocyte counts in millions/cu.mm. and reticulocyte percentages for the splenectomized animals of the splenectomy-laparotomy control series.

average value for the two animals at this time was 22.2 thousand cells/cu.mm. which is just slightly above the outer limits of normalcy. However, the percentage of lymphocytes in the differential counts had dropped from an average normal value of 81.7 per cent just prior to surgery to an average of 48.0 per cent at four days after surgery. In terms of absolute counts this means a decrease in the absolute lymphocyte count of 1.2 thousand cells/cu.mm. at the four day interval. The relative neutrophil count, however, had risen from 12.2 per cent just prior to surgery to 48.1 per cent at the four day interval. This means an absolute increase of neutrophils in the peripheral blood of 8.9 thousand cells/cu.mm. during this four day period following splenectomy. There was an increase in juvenile, staff and segmented forms of neutrophils but the greatest increase was in the more mature, segmented forms.

At eight days after splenectomy the total erythocyte count was still low, being an average of 2.4 million cells/cu.mm. However, the increase in the reticulocyte percentage at this time was very marked--the average for the two animals being 5[illegible].7 per cent. It was at around seven to eight days that most of the fatalities occurred following splenectomy. Just the opposite picture was seen regarding the leucocyte counts. At this time the average leucocyte count for these two animals was 41.1 thousand cells/cu.mm. However, the alterations in

the differential percentages seen at the four day interval had tended to disappear by eight days post surgery. The average lymphocyte count at this time was 70.1 per cent or an absolute increase in lymphocytes of 18.1 thousand cells/cu.mm. over the average value for the four day interval. The average neutrophil count at the eight day interval was 25.5 per cent or an absolute decrease of only 0.2 thousand cells/cu. mm. which is to be considered as no real change. Therefore, although the increase in the total leucocyte count for the first four days after splenectomy is made up intirely of neutrophilic leucocytes, the value for these elements doesn't change appreciably over the next four days but there is a sharp increase in total number of lymphocytes in the peripheral blood.

At fourteen days after splenectomy the red cell count had improved to a value of 5.2 million cells/cu.mm. for both animals. The percentage of reticulocytes had decreased from a value of 50.7 per cent at the eight day interval to one of 18.1 per cent at the fourteen day interval. Again the opposite trend was seen in the total leucocyte counts in respect to the erythrocyte counts. The total leucocyte count had fallen from an average value of 41.1 thousand cells/cu.mm. on the eighth day to 11.0 thousand cells/cu.mm. at fourteen days after splenectomy. This represents an absolute decrease of 20.9 thousand lymphocytes/cu.mm. and an absolute decrease

of 8.5 thousand neutrophils/cu.mm.

At twenty days after splenectomy, at which time the animals were sacrificed, the total red counts revealed another drop. The average total count at this time was 3.8 million cells/cu.mm. and the reticulocyte percentage was 28.0. Scrutiny of table IV will show that this decrease had occurred in both animals but was far more severe in one of them. The average total leucocyte count had risen again to a normal value of 18.0 cells/cu.mm. The differential percentages were not significantly different from those seen just prior to splenectomy. It will be pointed out later when the splenectomized control animals which were not bled at intervals that removal of sufficient blood from the tails of these animals plus the added insult of repeated anesthesia apparently had a detrimental effect as regards the anemia which these animals suffered following splenectomy.

In an effort to furnish comparison and possibly to cast some light on the causal relations of the anemia seen in the two previous animals following splenectomy a third animal was subjected to laparotomy, manipulation of the spleen and its pedicle but replacement of the spleen into the peritoneal cavity without ligation of its blood supply and separation from its pedicle. Peripheral blood studies were made at the same intervals as was described for the two splenectomized animals in this series. The blood picture just prior to

surgery (table V) was within the limits of normalcy for this rat colony. At the four day post-surgery interval the total red cell count had fallen from a pre-surgery value of 8.2 million cells/cu.mm. to 6.9 million cells/cu.mm. The reticulocyte count at this time is actually depressed indicating that strain on the bone marrow has not as yet had time to be reflected in the peripheral blood. The total leucocyte count is not elevated at this time nor is there any significant deviation in the differential leucocyte count from that seen before surgery. This same situation is true of the leucocyte picture at the eight day, fourteen day and twenty day intervals.

By eight days the total erythrocyte count had risen from 6.9 to 7.8 million cells/cu.mm. and the reticulocyte percentage had increased to 12.1 indicating an adequate response of the hematopoietic tissues to the blood loss of surgery and that removed for peripheral blood studies at the four day interval. From the eighth day until the twentieth day there was improvement in the total red cell count to a normal level and a decrease in the reticulocyte percentage to a normal level. Therefore, it was concluded that the severe anemia seen in the splenectomized animals was not due to blood loss, since that blood loss was almost identical for the splenectomized animals and the animal which underwent just a laparotomy.

Bone marrow: Bone marrow differential counts for all three animals in this series were determined at twenty days after

Days after Laparotomy	prior	4	8	14	20
Lymphocytes	77.0	77.3	79.3	70.0	72.7
Neutrophils	18.0	16.7	16.0	22.3	19.3
Segs	17.7	16.3	15.0	21.3	19.0
Staff	0.3	0.3	1.0	1.0	0.3
Juveniles	0	0	0	0	0
Eosinophils	2.7	3.3	2.7	5.7	3.0
Basophils	0	0	0	0	0
Monocytes	2.3	2.7	2.0	2.0	5.0
W.B.C.	17.5	17.7	19.5	16.9	10.0
R.B.C.	8.2	6.9	7.8	7.8	8.8
Reticulocytes	2.3	1.6	12.1	2.6	3.4

Table V. Differential leucocyte percentages, total leucocyte counts in thousands/cu.mm., total erythrocyte counts in millions/cu.mm. and reticulocyte percentages for the laparotomy animal of the splenectomy-laparotomy control series.

surgery (table VI). At this time all three show a relatively erythroblastic marrow, G/E ratios of 0.78/1.00 and 0.79/1.00 respectively for the splenectomized animals and 0.99/1.00 for the animal which underwent laparotomy only. Normal, it will be remembered, is circa 1.28/1.00 for the animals of this colony. Cut sections of bone marrow from the splenectomized animals (fig. 7) revealed a diffuse hyperplasticity of all elements but particularly striking are the numerous foci of erythropoiesis. The increase in megakaryocytopoiesis seen in these marrows would seem consistent with the finding of an increased platelet count so frequently described following splenectomy (Wintrobe, '51). Platelet counts were not done on these animals but empirically it was noted that there was always an apparent increase in the number of platelets to be seen in the blood smears made from splenectomized animals.

Lymph nodes and liver: These will be briefly mentioned only to say that no alterations from the normal configurations were in evidence in sectioned material from any one of the three animals in this series. There have been no reports in the literature either to indicate that these organs are particularly affected by splenectomy.

These observations are in accord with those of Lauda, '25, who noted that after removal of the spleen in the adult albino rat a severe anemia would develop in a few days to

Days after Splenectomy	20	-	
Days after Laparotomy	-	-	20
Granulocytic Cells	43.8	44.2	49.8
Neutrophils	38.2	33.6	37.8
Segs	6.4	4.0	12.6
Staff	17.0	14.6	14.2
Juveniles	10.8	9.2	7.4
Myelocytes	4.0	5.8	3.6
Eosinophils	4.0	5.0	8.4
Segs	0	0	0.2
Staff	1.2	1.2	3.2
Juveniles	1.6	2.2	3.0
Myelocytes	1.2	1.6	2.0
Basophils	0.2	0.6	0.4
Promyelocytes	0.8	3.6	1.8
Hemoblasts	0.6	1.4	1.4
Erythrocytic Cells	56.2	55.8	50.2
Basochromes	4.4	12.0	11.8
Polychromes	31.2	27.8	27.0
Orthochromes	20.6	16.0	11.4
Granuloid/Erythroid Ratio	0.78/ 1.00	0.79/ 1.00	0.99/ 1.00

Table VI. Values obtained from differential counts of touch preparations of femoral marrow from the animals of the splenectomy-laparotomy control series.

three weeks. The total erythrocyte count would drop from 8 to 10 million to 1 million cells/cu.mm. with a concommitant drop in the hemoglobin. Mayer, Borchardt and Kikuth, '26, reported finding Bartonella muris inclusion bodies in the red cells of rats following splenectomy. These appear as rods and diplococci in or on the red cells and appeared in large numbers at the heigth of the anemia. Marmorston-Gottesman and Perla, '30, found that in most colonies of albino rats B. muris is endemic or latent and that the adult normal rat is a carrier of the virus. These investigators noted that an aqueous lipoid extract of ox spleen would protect the albino rat against this disease following splenectomy or if a fourth or more of the spleen were left in situ the anemia would not develop. From their histological studies they reached the conclusion that the reticular cells play a specific role in protecting the rat against this virus. The rat is not the only animal in which removal of the spleen removes with it what is apparently a natural resistance to Bartonella infection. There is evidence to support the contention that oroya fever in man is found usually after a malarial infestation in which the spleen has been involved.

However, the existance of this infection in the colony of albino rats maintained by the Department of Anatomy has not as yet been definitely proven. In the splenectomized animals there is evidence of the presence of a hemolytic

anemia, i.e., drop in red cell count, reticulocytosis, macrocytosis, erythroblastic bone marrow and an elevated icteric index which was determined for one of the splenectomized animals at the time it was sacrificed; but no evidence of the inclusion bodies so adequately described in the literature as being a part of the picture of this disease could be found in any of the splenectomized rats. The existance of this disease being latent in this colony of rats then can only be presumed but at least it is known that splenectomy in these animals is followed by a severe hemolytic anemia which is accompanied by a high mortality rate. Splenectomy in albino rats which are known to be free of B. muris is followed by a slight, transitory anemia which apparently has no more significance than that seen in the animal of this series which underwent laparotomy only.

SPLENECTOMIZED SERIES (SERIES IV)

In this series four animals were splenectomized and subsequently sacrificed at eighteen, twenty-eight, thirty-eight and forth-eight days after surgery. No blood studies were conducted on these animals before they were sacrificed.

Peripheral blood: The animal which was sacrificed at the eighteen day interval showed evidences of having had a hemolytic affair in that the total erythrocyte count at this time was only 5.6 million cells/cu.mm. However, the reticulocyte percentage was within or very close to normal limits (table VII).

This probably indicated that the hemolytic condition which ravages so intensely following splenectomy as was demonstrated by the previous series has subsided by this time and the animal is well on the way to recovery. The lowred cell counts and the high reticulocyte percentages seen at the twenty day interval in the splenectomized animals of the preceding series more than likely can be blamed on the repeated insult of anesthetizing the animals and removing sufficient blood for the various studies which were carried out. In other words if it is assumed that a Bartonella muris infection is present in these animals, then these repeated insults lowered the animals' ability to combat and overcome the infectious process.

The total leucocyte count at the eighteen day interval is definitely elevated, 37.9 thousand cells/cu.mm., but this is to be expected in view of the findings for the preceding series and also in view of the findings of Palmer, Kemp, Cartwright and Wintrobe, '51, who noted that following splenectomy in the albino rat, the total leucocyte count increased approximately 100 per cent in seven days, and remained significantly elevated for seventy to ninety days. They report that the increase is made up of both neutrophilic polymorphonuclear and lymphocytes. This was pointed out for the splenectomized animals of the preceding series and is brought out again in the differential hemogram for the animals in this

Days after Splenectomy	18	28	38
Lymphocytes	76.0	74.0	68.3
Neutrophils	13.3	10.3	18.3
Segs	10.3	9.7	17.7
Staff	3.0	0.7	0.7
Juveniles	0	0	0
Eosinophils	2.7	11.3	9.7
Basophils	0.3	0	1.3
Monocytes	7.7	4.3	2.3
W.B.C.	37.9	12.8	16.1
R.B.C.	5.6	8.1	8.2
Reticulocytes	4.1	3.6	2.8

Table VII. Differential leucocyte percentages, total leucocyte counts in thousands/cu.mm., total erythrocyte counts in millions/cu.mm. and reticulocyte percentages for the animals of the splenectomized control series.

series which was sacrificed at eighteen days after splenectomy. This differential (table VII) shows a normal distribution of the cellular elements.

However, in this series of animals being reported the blood picture (table VII) was in no way significantly altered from that for control animals at the twenty-eight and thirty-eight day intervals after splenectomy. Unfortunately no blood was obtained for study from the animal sacrificed forty-eight days after splenectomy because of technical difficulties. No explanation for this inconsistency of findings is available at the present time.

Bone marrow: The most striking feature to be gleamed from the differential counts (table VIII) is the fact that the distribution is that of the control animals. The G/E ratios are all near the normal value of 1.28/1.00 and sectioned material revealed a normal appearing marrow at all of the intervals studied. This would seem to indicate that the hemolytic condition had been brought well under control and that the animals had nearly compensated for the effects of splenectomy by eighteen days after surgery and that compensation was, for all practical purposes, complete by twenty-eight days.

Lymph nodes and liver: Again these will be mentioned to state that no deviations from the normal pattern were in evidence in the sectioned material.

Before going on to the splenectomized radiophosphorus-

Days after Splenectomy	18	28	38	
Granulocytic Cells	56.6	58.6	54.4	51.2
Neutrophils	49.0	46.6	45.6	44.6
Segs	18.2	15.0	16.8	6.0
Staff	15.4	16.6	15.0	19.6
Juveniles	11.0	9.0	9.8	12.2
Myelocytes	4.4	5.6	4.0	6.8
Eosinophils	5.4	8.0	3.2	4.0
Segs	0.4	0.4	0.2	0
Staff	3.0	3.8	1.6	3.2
Juveniles	1.4	3.2	0.8	0.8
Myelocytes	0.6	0.6	0.6	0
Basophils	0	0.4	2.6	0
Promyelocytes	1.4	2.2	1.6	2.0
Hemoblasts	0.8	1.4	1.4	0.6
Erythrocytic Cells	43.4	41.4	45.6	48.8
Basochromes	9.8	12.0	9.0	6.8
Polychromes	25.0	18.2	24.0	30.2
Orthochromes	8.6	11.2	12.6	11.8
Granulcid/Erythroid Ratio	1.30/ 1.00	1.42/ 1.00	1.19/ 1.00	1.05/ 1.00

Table VIII. Values obtained from differential counts of touch preparations of femoral marrow from the animals of the splenectomized control series.

treated series, it would be well to briefly review the salient changes which are produced by radiophosphorus and those produced by splenectomy in the normal albino rat. Normal albino rats treated with 1.0 microcurie radiophosphorus per gram of body weight show a leucopenia with normal differential distribution, a depressed reticulocyte percentage but no anemia within the time interval studied, a relatively granuloblastic marrow early changing to a relatively erythroblastic marrow by six to eight days and progression to a more normal state after twenty days, slight hypoplasia of the bone marrow particularily evident at about fourteen days, increased ectopic erythropoiesis and giant cell formation in the spleen throughout the entire experimental period, and no evidence of cellular damage to spleen, lymph nodes or liver.

Splenectomy in rats of this colony is followed by a severe hemolytic anemia the effects of which persist for about three weeks, a reticulocytosis reaching 50 per cent and more, a leucocytosis reaching a 100 per cent increase by eight days but apparently returning to normal by three weeks, a neutrophilcytosis during the first four days with no increase in lymphocytes and a lymphocytosis during the period from four to eight days with no increase in neutrophils, hyperplastic, erythroblastic marrow returning to near normal by three weeks, and no evidence of any effect on the lymph nodes or liver.

SPLENECTOMIZED RADIOPHOSPHORUS-TREATED SERIES

(SERIES V)

Six animals were used in this series being sacrificed at two, six, eleven, fourteen, seventeen and twenty days after treatment with 1.0 microcurie radioactive phosphorus per gram of body weight or twenty, twenty-four, fifty-eight, thirty-two, sixty-four and thirty-eight days respectively after splenectomy.

Peripheral blood: Consideration will first be given to those animals sacrificed at two, six, fourteen and twenty days after radiophosphorus treatment because these animals were treated only eighteen days after splenectomy. It will be remembered that the animal in the splenectomized series which was sacrificed at eighteen days after splenectomy showed a total erythrocyte count of 5.6 million cells/cu.mm. (table VII), a normal reticulocyte percentage, a total leucocyte count of 37.9 thousand cells/cu.mm. and normal appearing bone marrow (table VIII). It was at this stage that the above mentioned animals of this series were injected with radiophosphorus. The animal sacrificed two days after radiophosphorus treatment had a total red cell count of only 5.2 million cells/cu.mm. and a reticulocyte percentage of 9.4 which must be considered as elevated (table IX). The total leucocyte count for this animal was 8.6 thousand cells/cu.mm. which is significantly below the average of 12 to 20 thousand for control

Days after Treatment	2	6	11	14	17	20
Days after Splenectomy	20	24	58	32	64	38
Lymphocytes	79.0	86.7	81.3	86.0	78.3	70.0
Neutrophils	16.0	10.7	12.7	11.7	17.7	26.3
Segs	14.7	10.0	11.0	10.3	15.3	25.7
Staff	1.3	0.7	1.7	1.3	2.3	0.7
Juveniles	0	0	0	0	0	0
Eosinophils	3.0	0.3	3.0	0.7	1.7	1.7
Basophils	0	0	0.3	0.3	0	0
Monocytes	2.0	2.3	2.7	1.3	2.3	2.0
W.B.C.	8.6	5.9	3.6	2.7	2.1	6.6
R.B.C.	5.2	4.0	9.0	6.0	8.3	5.0
Reticulocytes	9.4	32.3	5.6	3.3	3.2	4.7

Table IX. Differential leucocyte percentages, total leucocyte counts in thousands/cu.mm., total erythrocyte counts in millions/cu.mm. and reticulocyte percentages for the animals of the splenectomized radiophosphorus-treated series.

animals and sharply depressed in respect to the 37.9 thousand cells/cu.mm. seen in the splenectomized animal sacrificed at eighteen days after surgery. The differential hemogram for this splenectomized animal sacrificed two days after radiophosphorus treatment indicates a normal distribution (table IX).

The animal sacrificed at six days after radiophosphorus treatment (twenty-four days after splenectomy) showed a total erythrocyte count of 4.0 million cells/cu.mm. and a reticulocyte count of 32.3 per cent. The total leucocyte count for this animal was only 5.9 thousand cells/cu.mm. The animal of the previous, splenectomized series which was sacrificed at twenty-eight days after surgery had a total red cell count of 8.1 million cells/cu.mm., a reticulocyte percentage of 3.6 and a total leucocyte count of 12.8 thousand cells/cu.mm. (table VII). The difference here was related to the introduction of the radioactive phosphorus into the one animal. The non-splenectomized animal which was sacrificed at six days after treatment with radiophosphorus showed a total red cell count of 11.3 million cells/cu.mm., a reticulocyte percentage of 0.7 and a total leucocyte count of 6.7 thousand cells/cu.mm. (table II). The difference here was related to splenectomy.

The animal sacrificed and studied at fourteen days after treatment with radioactive phosphorus (thirty-two days after splenectomy) showed a red cell count of 6.0 million cells/cu.mm.,

a reticulocyte percentage of 3.3 and a total leucocyte count of 2.7 thousand cells/cu.mm. Again comparison should be made with the animal sacrificed at twenty-eight days after splenectomy only (table VII), i.e., a total erythrocyte count of 8.1 million cells/cu.mm., a reticulocyte percentage of 3.6 and a total leucocyte count of 12.8 thousand cells/cu.mm. The splenectomized radiophosphorus treated animal showed an anemia but a normal reticulocyte percentage and a markedly depressed total leucocyte count. The non-splenectomized animal sacrificed fourteen days after radiophosphorus treatment had a total erythrocyte count of 9.2 million cells/cu.mm., a reticulocyte percentage of 1.5 and a total leucocyte count of 5.6 thousand cells/cu.mm. (table II).

The animal sacrificed at twenty days after radiophosphorus treatment (thirty-eight days after splenectomy) had a total erythrocyte count of 5.0 million cells/cu.mm., a reticulocyte count of 4.7 per cent and a total leucocyte count of 6.6 thousand cells/cu.mm. In comparing this with the previous splenectomized only series we find that the animal sacrificed at thirty-eight days after splenectomy (table VII) had a total erythrocyte count of 8.2 million cells/cu.mm., a reticulocyte percentage of 2.8 and a total leucocyte count of 16.1 thousand cells/cu.mm. Again we see the inability of the radiophosphorus treated animal to regain a normal erythrocyte count and the continued depression but slight improvement of the leucocyte

count. The animal sacrificed twenty days after radiophosphorus treatment only had a total erythrocyte count of 7.7 million cells/cu.mm., a reticulocyte percentage of 0.6 and a total leucocyte count of 9.2 thousand cells/cu.mm. (table II).

The other two animals of the splenectomized radiophosphorus treated series were injected with radioactive phosphorus forty-seven days after splenectomy. The first one, sacrificed eleven days after treatment with radioactive phosphorus (fifty-eight days after splenectomy), had a total erythrocyte count of 9.0 million cells/cu.mm., a reticulocyte count of 5.6 per cent and a total leucocyte count of 3.6 thousand cells/cu.mm. (table IX). The non-splenectomized animal sacrificed at eleven days after treatment with radiophosphorus, unfortunately, underwent no peripheral blood studies. In the animal sacrificed seventeen days after radiophosphorus treatment (sixty-four days after splenectomy) the total erythrocyte count was 8.3 million cells/cu.mm., the reticulocyte count 3.2 per cent and the total leucocyte count 2.1 thousand cells/cu.mm. (table IX). In the animal sacrificed seventeen days after treatment with radiophosphorus the total erythrocyte count was 8.4 million cells/cu.mm., the reticulocyte count 3.7 per cent and the total leucocyte count 4.4 thousand cells/cu.mm. (table II).

It appears then that radioactive phosphorus administration to a splenectomized albino rat which has not completely re-

covered from the hemolytic anemia which follows splenectomy will tend to maintain the total erythrocyte level at the value obtained at the time of administration. Apparently this added insult prevents complete alleviation of the conditions producing the anemia. However, the depression of the reticulocyte count seen in non-splenectomized radiophosphorus treated rats is not seen in the splenectomized radiophosphorus treated animals. This observation is inconsistent with those of Jacobson, Simmons and Block, '49, who found that the reticulocyte values of mice which were splenectomized only and those which were given radiostrontium only had actually risen by seven days, whereas the reticulocyte value of mice which were both splenectomized and strontium-treated had fallen significantly. The one very significant point is the more marked depression of the leucocyte count seen in the splenectomized radiophosphorus treated animals with a normal differential distribution in both cases (tables II and IX). This is very interesting in view of the fact that the leucocyte count, of both polymorphonuclear and mononuclear, is so markedly elevated following splenectomy. The depression is more marked apparently without regard as to the length of time that has elapsed between splenectomy and radiophosphorus treatment.

Bone marrow: Here there is a quite definite relative increase in erythroblastic elements as seen in the G/E ratios for the

animals of this series (table X). The increase in erythroblasts, as with the non-splenectomized radiophosphorus treated animals, is in the later stages--polychromic erythroblasts and orthochromic erythroblasts. The major difference between the G/E ratios of the splenectomized radiophosphorus treated series and the non-splenectomized radiophosphorus treated series is to be seen within the first few days. The ratios for the non-splenectomized radiophosphorus treated animals which were sacrificed at two and four days reveal a preponderance on the granuloid side and a normal bone marrow picture respectively. The animal of the splenectomized radiophosphorus treated series sacrificed at two days revealed even at this time a preponderance of erythroid elements. From six days on to the twentieth day the two series of animals show an almost identical picture in so far as the differential counts are concerned. However, at the twentieth day the splenectomized radiophosphorus treated animal appeared to have had a relapse or else was compensating for a more severe degree of damage following splenectomy than was true of the remainder of the animals in this series because the marrow from this animal was very markedly erythroblastic whereas the marrow from the non-splenectomized radiophosphorus treated animal sacrificed at twenty days had just the opposite picture, i.e., predominately granuloid. Other animals given this dosage, 1.0 microcurie per gram of body weight, by other

Days after Treatment	2	6	11	14	17	20
Days after Splenectomy	20	24	58	32	64	38
Granulocytic Cells	48.6	33.8	35.6	29.4	45.0	22.2
Neutrophils	37.8	28.8	24.6	22.2	36.0	18.4
Segs	7.8	7.8	5.2	3.2	5.0	8.6
Staff	16.0	11.6	9.8	5.6	18.0	4.4
Juveniles	9.8	5.6	6.3	7.8	9.0	3.4
Myelocytes	4.2	3.8	3.3	5.6	4.0	2.0
Eosinophils	7.6	1.0	8.7	2.6	6.4	0.8
Segs	0.2	0	0.2	0	0	0
Staff	3.8	0.6	2.7	1.4	1.4	0
Juveniles	2.8	0.2	4.3	0.8	3.2	0.2
Myelocytes	0.8	0.2	1.5	0.4	1.8	0.6
Basophils	0.4	0	0.2	0	0	0
Promyelocytes	1.8	1.4	1.7	3.6	1.8	1.4
Hemoblasts	1.0	2.6	0.5	1.0	0.8	1.6
Erythrocytic Cells	51.4	66.2	64.4	70.6	55.0	77.8
Basochromes	9.6	12.8	9.7	11.4	12.6	14.4
Polychromes	24.0	44.0	35.5	36.6	32.8	42.8
Orthochromes	17.8	9.4	19.2	22.6	9.6	20.6
Granuloid/Erythroid Ratio	0.95/ 1.00	0.51/ 1.00	0.56/ 1.00	0.42/ 1.00	0.82/ 1.00	0.29/ 1.00

Table X. Values obtained from differential counts of touch preparations of femoral marrow from the animals of the splenectomized radiophosphorus-treated series.

workers (Latta and Kamprath, '51) and sacrificed at twenty days after treatment have consistently shown a G/E ratio to be near 1.00/1.00 indicating that the mechanisms for restoring the marrow to the normal functional level are adequately compensating for the radiation damage. The amount of radioactive phosphorus remaining in the bone cortex and cancellus protions at twenty days is small and probably of little significance (Latta and Vose, '50).

Cut sections of bone marrow are essentially similar for the two series. Comparison of the animals sacrificed at the fourteen day interval show increase in the number of sinusoids, engorgement of the s inusoids with blood, edema of the stromal areas with some destruction of the stromal pattern and diapedesis of red cells into the stromal areas. The actual cell population in both the sectioned material from the splenectomized radiophosphorus treated series fourteen day interval animal and the non-splenectomized radiophosphorus treated series fourteen day interval animal is decreased in volume over the normal configuration and erythroblastic in nature (fig. 4 and fig. 8). This is definite evidence of hypoplasia of bone marrow in both series. However, the severity of the hypoplasia in one does not seem to significantly differ from that seen in the other.

Jacobson, Simmons and Block, '49, found that extensive cellular depletion occurred in the bone marrow of mice given

2.0 microcuries radiostrontium per gram of body weight but that no peripheral anemia developed. However, if the same dosage were given to splenectomized mice a severe anemia would develop presumably because of the loss of the possibility for ectopic erythropoiesis in the spleen. They have supported this contention by lead shielding the mouse spleen while the remainder of the body is subjected to X-irradiation. Such a procedure obviated the development of anemia and significantly reduced the severity and the duration of the leucopenia and thrombocytopenia which regularly follow the delivery of the same dose to the whole body (Jacobson, Marks, Gaston, Robson and Zirkle, '49).

Were it purely a matter of the added quantity of myelopoiesis that the spleen is able to offer it would seem reasonable to expect that the reticulocyte counts in the non-splenectomized radiophosphorus treated animals would be elevated whereas in the splenectomized radiophosphorus treated animals they would be depressed. Just the opposite, however, was the case although it was shown that the splenectomized animals suffering from an anemia were unable to elevate their red cell counts after treatment with radiophosphorus as were untreated splenectomized animals during the same period of time but that splenectomized animals which had an adequate red cell count were not rendered anemic by radiophosphorus. The total leucocyte counts were consistently more depressed in the

splenectomized radiophosphorus treated animals than in the non-splenectomized radiophosphorus treated animals. This would seem to indicate either a protective or formative role of the spleen as regards these elements.

Lymph nodes and liver: In both of these organs examination of sectioned material did not reveal any effects secondary to splenectomy, radioactive phosphorus or a combination of both on either the cellular or gross structures.

DISCUSSION

This work has in many ways confirmed the observations reported by Jacobson and coworkers concerning the role of the spleen in hematopoietic recovery following radiation injury. There are certain inconsistencies, however, which should be analyzed more extensively. A prime example of this is the author's observation that reticulocyte counts in the rats treated with 1.0 microcurie radioactive phosphorus per gram of body weight are depressed 1 to 2 per cent below normal for at least the first two weeks after which there is a tendency to rise to normal by three weeks and to attain a 1 to 2 per cent increase over normal for the next two to three weeks. This was explained on the basis of a delay in reticulocyte delivery and maturation arrest which was apparently operative until the radiophosphorus concentration in the bone dropped below a certain level. However, Jacobson, Simmons and Block, '49, found that in mice treated with 2.0 microcuries radiostrontium per gram of body weight the reticulocyte percentage was elevated. Such an observation, it is true, would certainly be more in keeping with the facts that no anemia became apparent and that ectopic erythropoiesis became prominent in the spleens of the mice these authors investigated and also in the albino rats used for the work being reported herein.

Another example of inconsistent findings is the observation the author made of the normal to increased reticulo-

cyte counts in the splenectomized radiophosphorus treated animals as compared with the decreased reticulocyte counts which Jacobson, et. al., found in splenectomized radiostrontium treated mice. Also difficult to explain is the reticulocyte depression in the presence of ectopic erythropoiesis in the spleen whereas the radiophosphorus treated animals without a spleen showed no such depression.

Here one is forced to fall back to the earlier observations by Cruz and Robscheit-Robbins, '42, who found that in the splenectomized dog made anemic by bleeding or by acetylphenylhydrazine injections, the number of normoblasts in the peripheral blood was four times greater than in the non-splenectomized animal. Here as in the author's observations the interpretation of splenic inhibition on liberation of cells from the marrow becomes very inviting. Also the fact that the splenectomized animals in this work were suffering from a hemolytic anemia when injected with radiophosphorus which did not aggravate the severity of the anemia would seem to confirm studies by Jacobson, Marks, Gaston, Simmons and Block, '48, who found that X-irradiation of animals with regenerative anemia does not increase the severity of anemia. However, they found that these animals recover as soon as normal animals made anemic by the same dose of irradiation. The splenectomized albino rats which were anemic before

treatment with radiophosphorus did not recover from the anemia within twenty days. However, if the red cell count was normal in the splenectomized animal before radiophosphorus injection, no anemia became apparent. These data would tend to indicate that erythroblast vulnerability to irradiation injury is not enhanced by increased mitotic activity and proliferation. In fact, the hyperplastic erythroid tissue sustained less histologic injury than the normal, and the production of erythrocytes was maintained.

However, Jacobson, Marks, Robson, Gaston and Zirkle, '49, have reported that lead-protection of the marsupialized spleen from irradiation applied otherwise to the total animal, makes possible a rapid return to normal hematopoiesis in the bone marrow and protects the animal from total marrow aplasia and death. Here splenic tissue appears to be protective in nature rather than inhibitory. The fleeting thought that the spleen perhaps promotes bone marrow growth but tends to inhibit delivery is too easily disputed by the repeated observations of bone marrow hyperplasia following splenectomy (Krumbhaar, '32).

There are several points, however, on which the author and previous investigators reach agreement. Splenectomy in the albino rat if infected with Bartonella muris (which has not been proven for the animals of this colony) is followed by a severe hemolytic anemia with a high fatality rate but

by only a slight transitory anemia if not infected with B. muris (Perla and Marmorston, '35). This latter is well within the range of anemia produced by surgery alone. Splenectomy is also followed by a leucocytosis of over 100 per cent the first increase being in the polymorphonuclear population but being followed within a few days by an increase in the mononuclear population. Such a leucocytosis was present for only three weeks in the animals of my series but Palmer, Kemp, Cartwright and Wintrobe, '51, have reported such an increase to be present for seventy to ninty days following splenectomy in albino rats. These investigators reported that such an increase could be prevented if as little as 10 per cent of the spleen remained or if the splenectomized animal was in parabiotic partnership with a non-splenectomized animal. This they cited as being evidence in favor of splenic control of the rate of production and/or liberation of leucocytes in the bone marrow.

My observation that the total leucocyte count is depressed more severely in splenectomized radiophosphorus treated animals than in non-splenectomized radiophosphorus treated animals would seem to indicate that either the spleen was able to furnish a limited quantity of all types of leucocytes through natural lymphopoiesis and ectopic myelopoiesis or else the spleen exerted a sparing action on the bone marrow. In view of the reports by Palmer, et. al., the former explanation

would seem the most logical.

The mechanism and nature of the stimulus which induces ectopic erythropoiesis so rapidly in lead-protected spleens, or spleens of animals subjected to radiophosphorus or radiostrontium treatment are not clear since no anemia or other known stimulus of erythropoiesis appears to exist. Jacobson, et. al., have suggested that perhaps the oxygen saturation of the circulating blood is temporarily interfered with or that destruction of hematopoietic tissue itself may produce the stimulus. On the other hand the fact that the bone marrows, the thymi, the lymph nodes, and the patches of Peyer of Jacobson's animals which had spleen protection were never depleted of free cells and returned to normal cellularity more quickly than did these tissues in animals without spleen protection is replete with more interesting possibilities for speculation.

Irradiation of the spleen (which would be slight in radiophosphorus or radiostrontium administration) may alter the spleen to the extent that it functions as a pathologic organ and by some humoral mechanism suppresses hematopoietic regeneration during this critical phase of radiation toxicity.

Another and perhaps more likely possibility is that protection of the spleen simply provides the animal with a normal but hyperactive spleen which acts in some way to reduce "radiation toxicity" to the extent that the normal regenerative

ability of hematopoietic tissue is permitted to proceed.

Since lead-protection of either the spleen or the appendix of the rabbit during irradiation allows a more rapid regeneration of the irradiated hematopoietic tissue than in rabbits without such protection, it is obvious that neither is it the spleen specifically nor the ability of the lead-protected tissue to produce ectopic blood formation that accounts for the observed phenomenon. These findings strongly suggest that lymphatic tissue exerts a humoral control over hematopoiesis. It might be postulated that the bone marrow which is not normally lymphopoietic, and the thymus would produce a comparable effect were it possible to test this postulate by exclusive protection of these tissues during irradiation. Such experiments are probably necessary before one can assume that the entire hematopoietic system functions as a unit in the maintenance of a steady state. Colonization or seeding from the lead protected lymphatic tissue may actually provide cells from which the irradiated marrow and lymphatic tissue become repopulated. If this latter suggestion were true, then it would follow that the lymphocytes coming from a protected lymphatic organ such as the appendix and going to the bone marrow were capable of giving rise to all types of myelopoietic cells.

If the irradiated lymphatic tissue is acting to temporarily suppress hematopoietic regeneration, then the process

may be related to the "indirect" therapeutic effect observed in myelogenous leucemia when the spleen alone is irradiated.

Whether the factor involved is due to cell migration from the shielded tissue or dispersion of a non-cellular substance is not yet proved. The evidence presented points in the direction of a humoral non-cellular substance which stimulates regeneration of the hematopoietic tissue or supplies something which makes repair and regrowth possible. The possibility that irradiation produces a "toxin" which inhibits regeneration of irradiated tissue and that the shielded tissue neutralizes such a toxin has not been entirely disproved.

Thus the possibility that the spleen, which is an organ in which blood cell formation, blood cell sequestration and destruction, and possibly hormone production occurs, can stimulate irradiated tissue to an earlier recovery or compensate for damaged hematopoietic tissues is incouraging as a possibility of post-irradiation "therapy". The potential significance of these findings to the problem of radiation injury and to certain other clinical syndromes is thought provoking.

SUMMARY AND CONCLUSIONS

1. An attempt has been made to determine in some measure the effect of splenectomy on recover from radioactive phosphorus radiation injury. Five series of albino rats were used--a control series, a non-splenectomized radiophosphorus treated series, a splenectomized radiophosphorus treated series and two control splenectomized series. Radiophosphorus treated animals were injected with 1.0 microcurie per gram of body weight. Both splenectomized and non-splenectomized radiophosphorus treated series were studied over a period of twenty days.

2. The control splenectomized series revealed splenectomy in this colony of albino rats to be followed by a severe hemolytic anemia, an immediate increase in polymorphonuclear cells followed within a few days by an increase in mononuclear cells to give a leucocytosis of over 100 per cent which persisted for circa three weeks. The leucocyte count then returned to normal with a normal differential picture. The platelets were estimated empirically to be increased. The reticulocyte counts increased to over 50 per cent by eight days with a return to normal at about three weeks and the appearance of a normal red count between three and four weeks. Bone marrow revealed erythroblastic hyperplasia.

3. Non-splenectomized animals given 1.0 microcurie radiophosphorus per gram body weight showed a definite depression

in the number of leucocytes, although the differential count was not appreciably altered, and also a depression of the reticulocyte percentage except at seventeen days after treatment. In the bone marrow there was a progressive "shift to the right" in the erythroblastic series up to seventeen days a relative or absolute decrease in the number of granulocytes, and in most cases a slight degree of hypoplasia accompanied by a loss of the normal arrangement of the sinuses and vascular channels. There was apparently a reticulocyte shower at about the sixteenth or seventeenth day disrupting the granulocytic/erythroblastic ratio and producing a great preponderance of granulocytes. The spleen had foci of erythropoiesis and large rounded up macrophages present by two days and evident during the entire twenty day experimental period. No evidence was found of any lymphocyte damage except for the peripheral lymphopenia. The liver and lymph nodes showed no reaction to the radioactive phosphorus.

4. Splenectomized animals given 1.0 microcurie radiophosphorus per gram of body weight did not reveal any depression in the reticulocyte count and even evidenced a reticulocytosis. The radiophosphorus was administered to two thirds of the animals before they had recovered completely from the hemolytic anemia which followed splenectomy and in these animals the total erythrocyte count never attained a value above 6.0 million cells/cu.mm. this being approximately the value at

the time of the administration of the radiophosphorus. In splenectomized animals which had attained a normal red count there was no depression seen following radiophosphorus treatment. The leucocyte counts were markedly depressed below the level seen in the non-splenectomized radiophosphorus treated animals. Differential distribution was normal. The progressive shift to the right seen in the marrows of non-splenectomized radiophosphorus treated animals is also seen in splenectomized radiophosphorus treated animals and appears four days earlier in the experimental period. Otherwise the bone marrows from the splenectomized radiophosphorus treated animals appeared almost identical with the bone marrow of the non-splenectomized radiophosphorus treated animals. The liver and lymph nodes here show no reaction ot the radioactive phosphorus either.

5. Although the bone marrow, spleen, and lymphatic tissues are of the same order in respect to radiosensitivity, the bone marrow is the most heavily affected by radioactive phosphorus intoxication because of the biological properties of phosphates as chemical compounds. Radiation damage to hematopoietic organs, as with other tissues, is manifested as cellular destruction and inhibition of mitosis which give rise to conditions varying from slight hypoplasia to aplasia, the picture being roughly proportional to the administered dosage. Such damage to the hematopoietic organs, if severe enough,

will be reflected in the peripheral blood as a pancytopenia. Even though the bone marrow has been subjected to a dose of radiation capable of producing aplasia, if the spleen has been relatively free from radiation as with radiophosphorus or radiostrontium intoxication or lead shielding of the spleen this organ is capable of favorably affecting recovery by ectopic myelopoiesis and by the dispersion of either cellular elements or humoral substances which are capable in some manner of producing rapid return of damaged hematopoietic tissue to the normal state. The preponderance of evidence seems to indicate that ectopic myelopoiesis plays at least a minor role, that cellular dispersion of lymphocytes from the spleen or other protected lymphatic organs to the damaged hematopoietic organs is probably negligent, and that the possibilities of a humoral mechanism eminating from the spleen affecting a rapid recovery of hematopoietic tissues is likely the most important factor in recovery from radiation injury. The obvious connection this could have to therapeutic measures in radiation injuries is intriguing.

6. Splenic functions have been briefly reviewed.

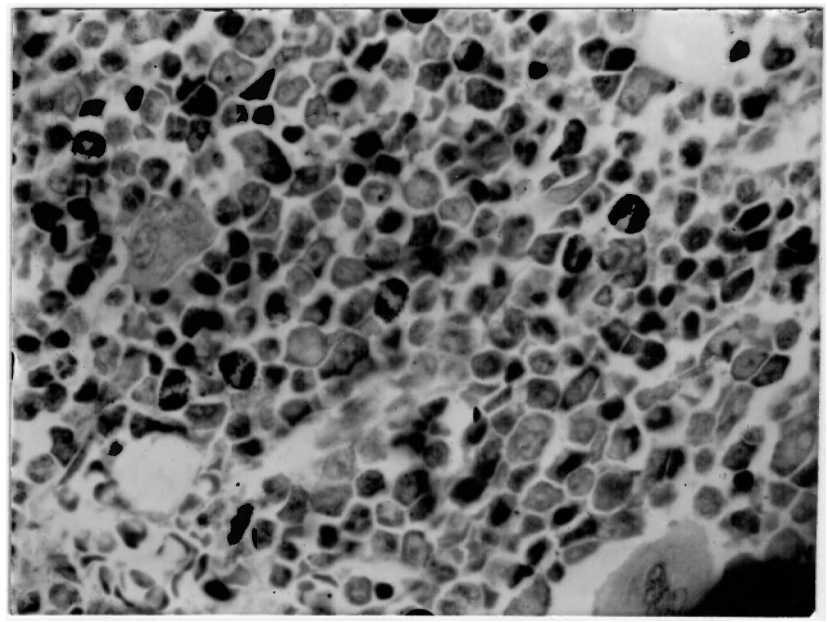

Figure 1. Bone marrow from an untreated animal. A normal stromal pattern is apparent densely packed with hematepoietic cells. One blood sinusoid, two fat cells, and two megakaryocytes are shown representing normal configuration.

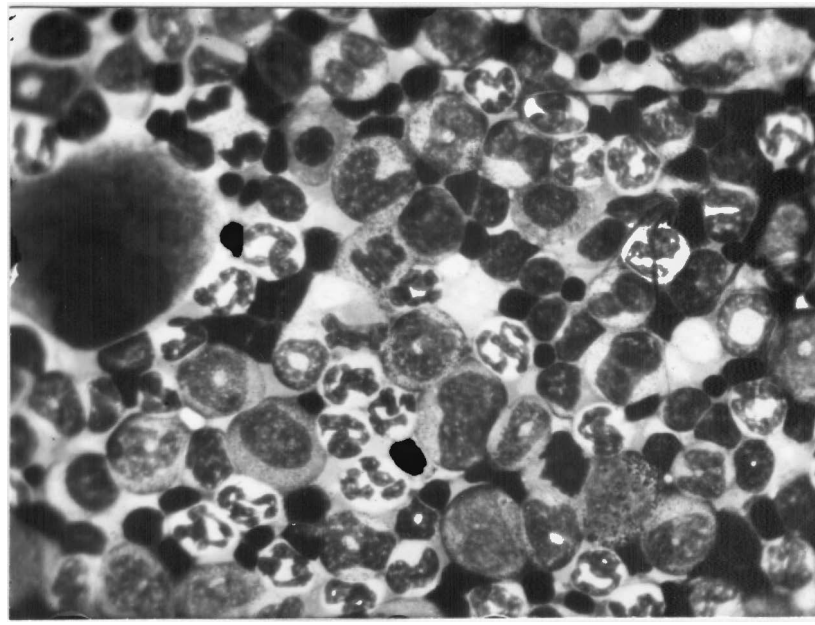

Figure 2. Bone marrow imprint from one of the control animals. Note the relative distribution of granuloid and erythroid elements. This is typical of the marrows taken from control animals.

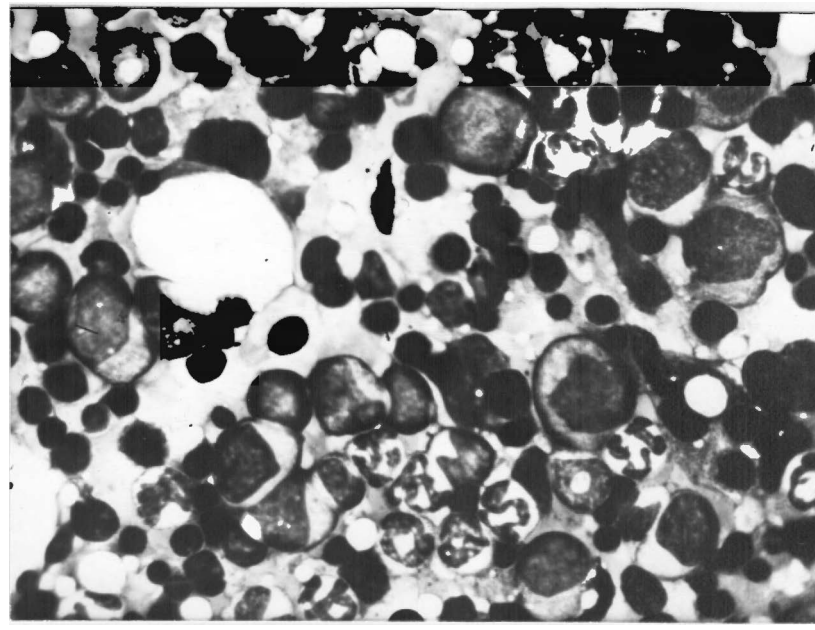

Figure 3. Bone marrow imprint fourteen days after treatment with 1.0 microcurie radioactive phosphorus per gram body weight. The number of mature erythroblasts is much greater than normal. Cellular details do not appear disturbed.

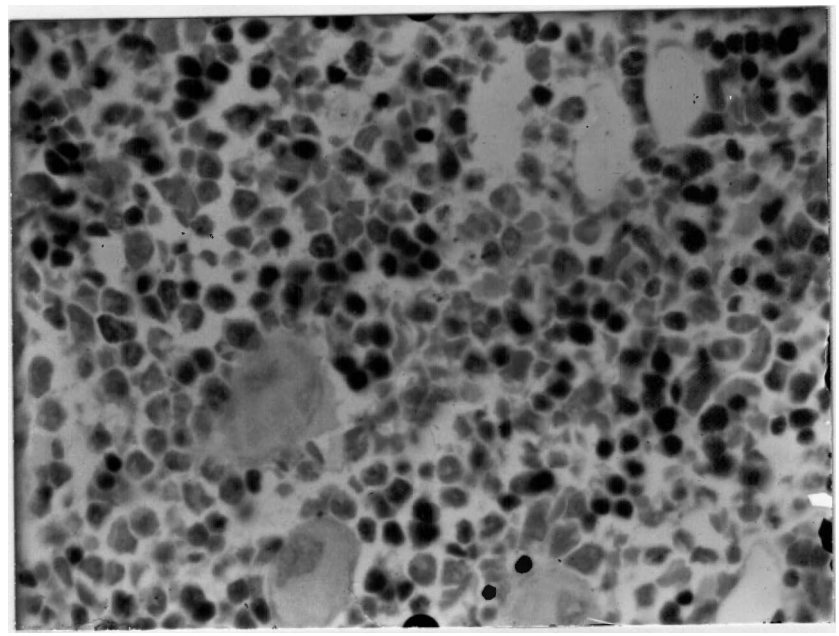

Figure 4. Bone marrow fourteen days after treatment with 1.0 microcurie radioactive phosphorus per gram body weight. The stroma is loose and congested with mature erythrocytes. The number of fat cells and megakaryocytes is slightly increased and the number of erythropoietic foci is markedly increased.

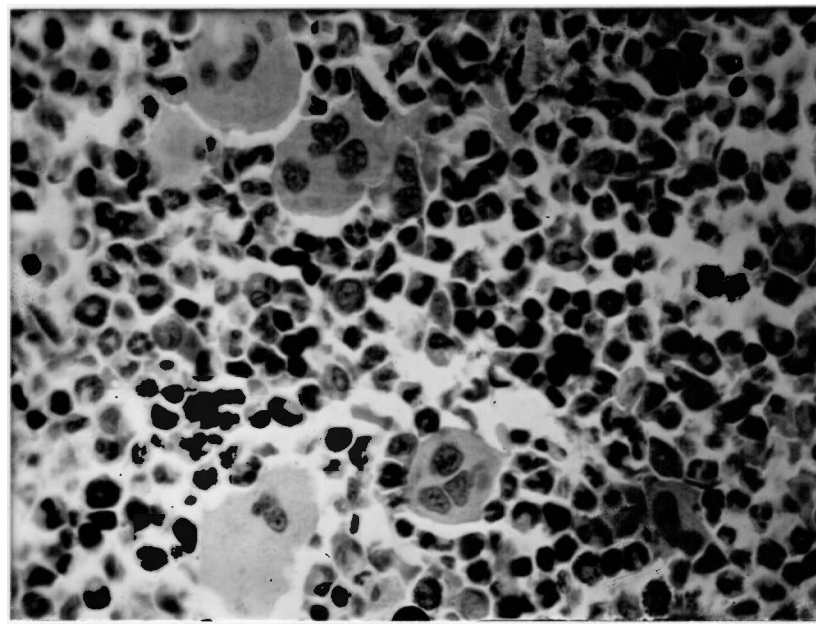

Figure 5. Bone marrow twenty days after treatment with 1.0 microcurie radioactive phosphorus per gram body weight. Note the dense cellular structure of the stroma, the increased number of megakaryocytes, and the sparsity of erythropoietic foci, fat cells and sinusoids.

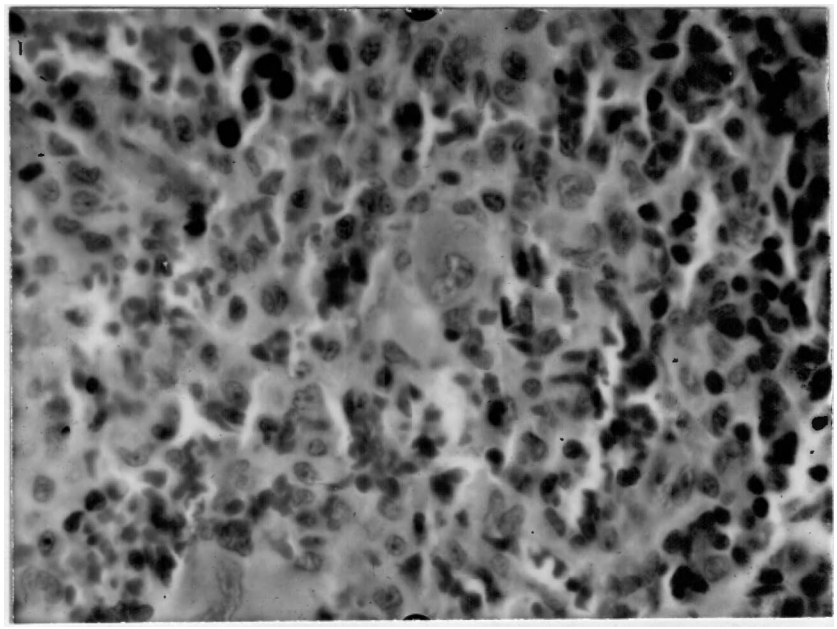

Figure 6. Spleen twenty days after treatment with 1.0 microcurie radioactive phosphorus per gram body weight. Only the edge of the outer zone of white pulp is shown. Note the increased number of erythropoietic foci and the large rounded up macrophages in the red pulp.

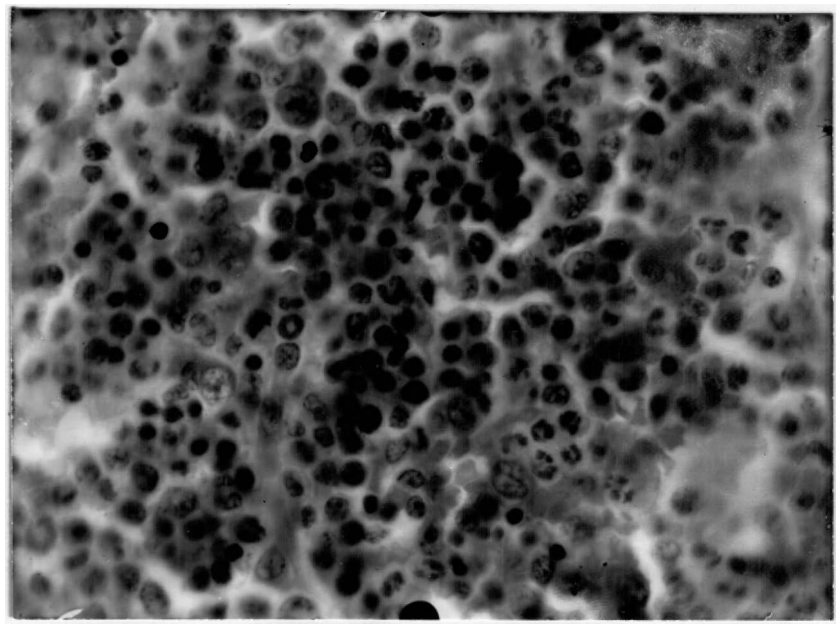

Figure 7. Bone marrow from a splenectomized animal of the splenectomy-laparotomy series. Note the dense packing of hematopoietic cells in the normal stroma with a preponderance of erythroblastic elements.

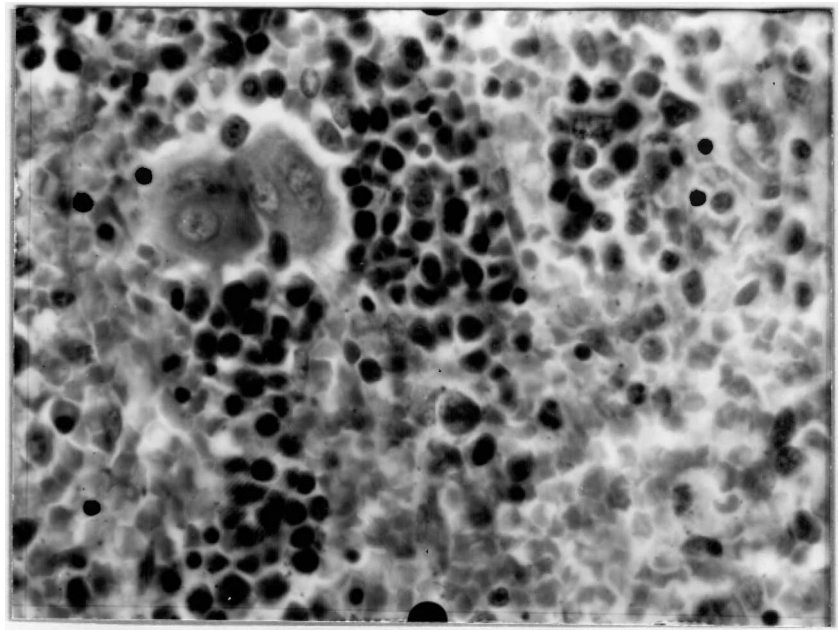

Figure 8. Bone marrow from a splenectomized animal fourteen days after treatment with 1.0 microcurie radioactive phespherus. The normal stromal features are disturbed with edema and congestion of stromal areas with erythrocytes. The number of sinusoids is increased but the number of fat cells are not. The number of erythroblastic foci vastly predominate the granuloblastic foci.

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